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Canine sarcomas as a surrogate for the human disease

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Abstract

Pet dogs are becoming increasingly recognized as a population with the potential to inform medical research through their treatment for a variety of maladies by veterinary health professionals. This is the basis of the One Health initiative, supporting the idea of collaboration between human and animal health researchers and clinicians to study spontaneous disease processes and treatment in animals to inform human health. Cancer is a major health burden in pet dogs, accounting for approximately 30% of deaths across breeds. As such, pet dogs with cancer are becoming increasingly recognized as a resource for studying the pharmacology and therapeutic potential of anticancer drugs and therapies under development. This was recently highlighted by a National Academy of Medicine Workshop on Comparative Oncology that took place in mid-2015 (http://www.nap.edu/21830). One component of cancer burden in dogs is their significantly higher incidence of sarcomas as compared to humans. This increased incidence led to canine osteosarcoma being an important component in the development of surgical approaches for osteosarcoma in children. Included in this review of sarcomas in dogs is a description of the incidence, pathology, molecular characteristics and previous translational therapeutic studies associated with these tumors. An understanding of the patho-physiological and molecular characteristics of these naturally occurring canine sarcomas holds great promise for effective incorporation into drug development schemas, for evaluation of target modulation or other pharmacodynamic measures associated with therapeutic response. These data could serve to supplement other preclinical data and bolster clinical investigations in tumor types for which there is a paucity of human patients for clinical trials.

Keywords

Sarcomas; Hemangiosarcoma; Osteosarcoma; Canine; Drug development; Comparative oncology

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Conflict of interest

The authors declare no conflicts of interest.

1. Introduction

Neoplasia is the leading pathophysiologic process responsible for death in 70 of 81 dog breeds and in mixed-breed dogs in North America. The breeds in which cancer is responsible for >40% of deaths include the Bernese Mountain Dog (55%), Golden Retriever (50%), Scottish Terrier (48%), Bouvier des Flandres (47%), Boxer (44%), Bullmastiff (44%), Irish Setter (41%), and Airedale Terrier (40%). Breeds with a low prevalence of cancer caused death include the Maltese (9%), Dachsund (9%), Pekingese (8%), Pomeranian (8%), Chihuahua (8%), Miniature Dachsund (6%) and Miniature Pinscher (4%). Standard breed height or weight correlates with the frequency of deaths from cancer (Fig. 1); the average weight of the breeds with a >40% cancer-caused deaths is approximately 31 kg whereas the average weight of the breeds with a <10% cancer-caused deaths is 5 kg. Overall, it is estimated that ~30% of dogs die from cancer, with the data from purebreed dogs showing 27.2% and an analysis of nearly 18,000 mixed-breed dogs showing 27.6% (Fleming, Creevy, & Promislow, 2011). These numbers reflect not only the large cancer burden in dogs but also reveal a population of spontaneously arising tumors whose treatment may be incorporated into cancer research and drug development strategies as an advanced surrogate prior to or in coordination with human trials.

The treatment of dogs with cancer is largely quite similar to humans, with the “triumvirate” of surgery, radiation therapy and chemotherapy forming the mainstay of treatment options. Surgical approaches are generally more conservative than those used in humans, and radiation and chemotherapy dose intensity is generally reduced compared to regimens in humans. This may be responsible in part for the inferior clinical outcomes generally observed in dogs versus humans. Recently, a limited arsenal of “targeted” agents and immunotherapies has become available in veterinary oncology, but the available agents are much more limited compared to human treatment options. The majority of medical therapies used for canine cancer treatment are older therapies for which generics are available. Newer agents are rarely used clinically, largely owing to cost constraints and a lack of dosing, safety, and efficacy information in dogs.

2. Sarcomas in dogs

Sarcomas make up approximately 10–15% of malignant tumors in dogs, with 20% of these tumors originating in the bone and the other 80% representing soft tissue sarcomas (STS). The total number of canine sarcomas occurring in the United States annually is estimated to be 7700 to 31,800 based on an estimated overall cancer incidence of 99.3–272.1 per 100,000 dogs (Merlo et al., 2008) and a canine population in the US of 78 million. The relative incidence and estimated incidence in the US of specific sarcomas in the dog are shown in Table 1. The major non-STS are represented by osteosarcoma (OSA) and chondrosarcoma (CSA). The primary STS in dogs are hemangiosarcoma (HSA), fibrosarcoma (FSA), peripheral nerve sheath tumors (PNST) and histiocytic sarcoma (HS), with myxosarcoma (MYX), liposarcoma (LIP), rhabdomyosarcoma (RMS), leiomyosarcoma (LMY), synovial cell sarcoma(SCS) and lymphangiosarcoma (LYA) occurring with much lower incidence (Bastianello, 1983; Dorn, Taylor, Schneider, Hibbard, & Klauber, 1968; Gruntzig et al.,

2016; MacVean, Monlux, Anderson, Silberg, & Roszel, 1978). For comparison, it is estimated that there will be 12,390 STS diagnosed in adults and children in the United States in 2017 and approximately 200 cases of OSA in children (Society, 2017).

2.1. Overview of canine soft tissue sarcomas

Hemangiosarcoma represents one of the primary STS in dogs and occurs primarily in the spleen, heart, liver, and the skin and subcutis (Brown, Patnaik, & MacEwen, 1985; Hargis, Ihrke, Spangler, & Stannard, 1992; Oksanen, 1978; Priester, 1976; Schultheiss, 2004; Srebernik & Appleby, 1991; Ward, Fox, Calderwood-Mays, Hammer, & Couto, 1994; Ware & Hopper, 1999). Hemangiosarcoma occurs more frequently in the Shepherd and Boxer dog and has also been reported to be overrepresented in Labrador and Golden Retrievers (Brown et al., 1985; Gruntzig et al., 2016; Schultheiss, 2004; Srebernik & Appleby, 1991). Hemangiosarcoma is an aggressive and highly metastatic tumor in the dog and can present with clinical signs ranging from vague, nonspecific illness to acute death from tumor rupture and massive blood loss.

Fibrosarcoma is roughly as prevalent as HSA in dog and arises from transformed fibroblasts primarily in the skin, subcutaneous space and oral cavity. Fibrosarcoma seems to be more prevalent in Dobermans, Rottweilers and Setters (Gruntzig et al., 2016). While locally infiltrative and prone to recurrence, metastasis is observed in approximately 20% of cases (Ciekot et al., 1994).

Peripheral nerve sheath tumors (PNST) have been previously termed neurofibrosarcoma, malignant Schwannoma and hemangiopericytoma, but generally all of these are currently regarded as having nerve sheath origin and are classified as PNST (Chijiwa, Uchida, & Tateyama, 2004). These tumors can occur anywhere in the body and are classified as *peripheral* (away from the brain and spinal cord), *root* (directly adjacent to the brain or spinal cord), or *plexus* (adjacent to the brachial or lumbosacral plexus) with the *peripheral* form having the most favorable treatment outcomes (Brehm, Vite, Steinberg, Haviland, & van Winkle, 1995). A recent retrospective analysis suggests that PNST does not have strong breed prevalence, but German Shepherds may be more susceptible as well as females (Boos et al., 2015). Progression of PNST is usually due to local recurrence and invasion, with distant metastasis occurring less frequently.

Histiocytic sarcoma (HS) originates from antigen-presenting dendritic cells and was originally recognized in Bernese Mountain dogs (Rosin, Moore, & Dubielzig, 1986) where inheritance has been identified (Padgett, Madewell, Keller, Jodar, & Packard, 1995). A variety of primary sites have been observed. Histiocytic sarcoma is aggressive and spreads to lymph nodes, kidneys, liver and the central nervous system. A hemophagocytic form of HS exists that causes anemia, hypoalbuminemia, thrombocytopenia and leukopenia and generally results in poor outcomes (Moore, Affolter, & Vernau, 2006).

Other more rare but distinct sarcoma sub-types observed in dogs include those that primarily arise in the subcutis including LIP, LYA, MYX, RMS, and SCS, and those arising primarily in the gastrointestinal tract which include gastrointestinal stromal tumor (GIST) and LMY. While LIP represents the most common subtype of human STS, these tumors are rare

neoplasms in the dog, and display differences in their anatomical distribution as compared to their human counterparts. Canine LIP most commonly arises within the subcutis (Baez, Hendrick, Shofer, Goldkamp, & Sorenmo, 2004; Dennis et al., 2011), which is in contrast to human LIP, in which these tumors are predominately located in the deeper soft tissue and musculature of the extremities, or for certain histological subtypes (dedifferentiated liposarcoma), predominately within the retroperitoneal space (Fletcher, Bridge, Hogendoorn, & Mertens, 2013). However, a recent study demonstrated that similar to human LIP, canine LIP also shares molecular aberrations in MDM2 and CDK4 (Avallone et al., 2016), suggesting potential value in evaluation of cell cycle-targeted therapeutics in dogs with LIP.

Rhabdomyosarcoma represents an aggressive form of human STS, with continued need for the development of additional therapeutic agents. In dogs, the diagnosis and classification of canine RMS into subtypes and variants is based entirely on histological features that closely parallel those classification schemes used in human medicine (Cooper & Valentine, 2008; Parham, 2001). However, additional comparative studies on the molecular pathogenesis of this disease in dogs are necessary, as while chromosomal translocations involving the *PAX3/7* and *FKHR* genes are prognostically important molecular features implicated in the pathogenesis of human RMS (Sorensen et al., 2002), to date no cytogenetic abnormalities have been reported in canine RMS.

Similar to human gastrointestinal sarcomas, LMY and GIST are two neoplastic entities which also occur in the gastrointestinal tract of dogs, and whose significant histological similarity requires the use of IHC for their differentiation. Canine LMY are characterized by positive labeling for desmin and smooth muscle actin, and negative labeling for KIT, while immunoreactivity for KIT confirms a diagnosis of GIST, regardless of reactivity to desmin or SMA (Dailey, Ehrhart, Duval, Bass, & Powers, 2015; Hayes, Yuzbasiyan-Gurkan, Gregory-Bryson, & Kiupel, 2013; Russell et al., 2007). The distinction between canine LMY and GIST is clinically important, as similar to humans, a subset of canine GISTs are also reported to have activating mutations in exon 11 of the *c-kit* gene, potentially rendering these tumors sensitive to receptor tyrosine kinase inhibitors (Gregory-Bryson, Bartlett, Kiupel, Hayes, & Yuzbasiyan-Gurkan, 2010).

While SCS represents a common STS of the extremities in humans, canine SCS is a rare and relatively poorly characterized neoplasm (Cagle, Mirra, Storm, Roe, & Eilber, 1987; Pool & Thompson, 2008; Vail et al., 1994). Initially considered to be the most common tumor of the canine joint, the advent of routine IHC has led to improved histological differentiation of canine joint sarcomas, with subsequent retrospective studies demonstrating that in fact, most articular/periarticular sarcomas in dogs are HS, further increasing the difficulty and confounding the comparative value of studying this tumor in dogs (Craig, Julian, & Ferracone, 2002). Lastly, canine LYA is a tumor of lymphatic endothelial origin which occurs primarily in large breed dogs, with affected sites typically including the dermal and subcutaneous tissues of the limbs, axilla, ventral cervical and inguinal regions, thorax, and abdomen (Curran, Halsey, & Worley, 2016; Williams, 2005). In contrast to humans, in which chronic lymphedema of years duration almost invariably precedes the development of LYA, dogs with LYA present with a more acute course of swelling and edema, typically as a sequela to tumor growth (Curran et al., 2016; Kaufmann, Chu, & Kaufman, 1991; Sharma &

Schwartz, 2012; Williams, 2005). Similar to SCS, canine LYA is also a rare neoplasm in the dog, with only sporadic case reports and a single 12 dog case series having been described in the literature since 1981 (Kelly, Wilkinson, & Allen, 1981), undermining the translational value of pet dogs with this tumor type.

2.2. Overview of canine non-soft tissue sarcomas

Osteosarcoma is the most common primary tumor of the bone in dogs. It is derived from primitive bone cells occurring in both the appendicular (~75%) and axial (~25%) skeleton (Brodey & Riser, 1969; Heyman, Diefenderfer, Goldschmidt, & Newton, 1992). The breeds with the highest incidence in the United States are the Saint Bernard, Great Dane, Doberman Pinscher, Irish Setter, Rottweiler, German Shepherd, and Golden Retriever. Although there does seem to be a hereditary basis for OSA based on breed and familial incidence, the major predisposing factor is the size of the dog with height being a stronger predictor than weight (Ehrhart, Ryan, & Fan, 2013). Osteosarcoma is highly metastatic, with metastasis arising early in the disease course and the lung being the primary site of spread (Spodnick et al., 1992).

Chondrosarcoma (CSA) is a primary tumor of the bone originating from chondrocytes and is the second most common bone tumor in dogs (Brodey, Misdorp, Riser, & van der Heul, 1974). Chondrosarcoma can arise in many primary sites, and the Golden Retriever seems to be at the highest risk (Popovitch, Weinstein, Goldschmidt, & Shofer, 1994). Chondrosarcoma is not particularly aggressive, with metastasis slow to develop although this may be dependent on the site of the primary tumor (Sylvestre, Brash, Atilola, & Cockshutt, 1992).

2.3. Potential advantages of spontaneous canine sarcomas for translational studies in human cancers

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. Dogs with spontaneous tumors naturally develop therapy resistance and spontaneous metastasis. Canine tumor burdens are similar to humans, which may be important with regard to biological factors such as hypoxia and clonal variation. The ease with which serial sampling of tumor tissue can be performed in dogs allows the collection of data that would be very challenging in humans (e.g. measurement of tumor molecular PD endpoints and intratumor drug concentrations, therapy-induced changes in anti-tumor immune responses, invasive validation of noninvasive imaging-based endpoints). This is due in part to the fact that these clinical patients are routinely sedated/anesthetized for these procedures, mitigating owner concerns regarding patient discomfort. The relatively common occurrence of these tumors in dogs versus humans affords a unique opportunity for translational research in tumor histotypes that may be extremely challenging to study in humans (e.g. OSA, HSA, HS). The fact that these tumors arise spontaneously in immunocompetent hosts and share similar degrees of intratumor heterogeneity (including presumably antigenic heterogeneity) allows for the evaluation of novel immune-based therapy approaches in a model that is likely to be a more faithful representation of the human disease. Finally, the lack of an established standard of care for many canine sarcomas

facilitates the rapid evaluation of novel therapies in contexts where they may be more efficacious (e.g. the postoperative “minimal residual disease” setting, in combination with other modalities) comparatively early in the drug development timeline (Paoloni & Khanna, 2008; Vail & Thamm, 2004).

3. Hemangiosarcoma

3.1. Comparative pathology of canine hemangiosarcoma

Comparatively, canine HSA is similar to the angiosarcoma sub-group of human STS (Fosmire et al., 2004). While these tumors share a somewhat similar anatomical distribution in both species, with the potential to arise in cutaneous, subcutaneous, and visceral sites, almost half of angiosarcomas in humans are cutaneous, arising in the head and neck region (Antonescu, 2014; Fury, Antonescu, Van Zee, Brennan, & Maki, 2005). In contrast, visceral HSA is more commonly observed than the cutaneous form in dogs (Schultheiss, 2004). Historically, canine HSA was presumed to arise from transformed vascular endothelial cells based primarily on the histomorphological appearance of the tumor. However, more recent genomic investigations into the nature of HSA in both dogs and humans suggest that these neoplasms likely originate from hematopoietic endothelialprogenitor cells, potentially of three different lineages (Gorden et al., 2014).

Neoplastic cells of canine HSA are highly pleomorphic, polygonal to spindle-shaped cells reminiscent of other canine sarcomas, but are distinguished by their distinct formation and lining of irregular, anastomosing vascular spaces, varying in size from capillary-like to cavernous (Kim, Graef, Dickerson, & Modiano, 2015; Prymak, McKee, Goldschmidt, & Glickman, 1988) (Fig. 2A). As observed in human angiosarcoma, canine HSA tumors can also appear as less differentiated, solid sheets of cells with epithelioid morphology and devoid of vasoformative structures, sometimes requiring their differentiation from carcinomas through the use of immunohistochemical (IHC) markers. Histological tumor grading of canine HSA has been defined and is based on overall differentiation, cellular pleomorphism, and necrosis (Ogilvie, Powers, Mallinckrodt, & Withrow, 1996). However, as is the case for grading schemes utilized in human cutaneous angiosarcoma, the prognostic significance of this grading scheme is under debate (Dettenborn et al., 2014).

Immunophenotypically, canine HSA expresses many of the same vascular endothelial markers as human angiosarcoma. These markers can aid in the diagnosis and distinction of HSA from other canine sarcomas, and include CD31, Factor VIII-related antigen (F8RA), and CD34 (Ferrer, Fondevila, Rabanal, & Vilafranca, 1995; Sabattini & Bettini, 2009; von Beust, Suter, & Summers, 1988). Additional IHC studies of canine HSA have also demonstrated the expression of other signal transduction proteins of potential therapeutic significance including CD117 (c-KIT), VEGFR1, VEGFR2, VEGFR3, and FGFR1 (Sabattini & Bettini, 2009; Yonemaru, Sakai, Murakami, Yanai, & Masegi, 2006). Importantly, cutaneous angiosarcomas of vascular and lymphatic origin can be distinguished in dogs based on expression in the latter of the lymphatic markers lymphatic vessel endothelial receptor-1 (LYVE-1) and prospero-related homeobox gene 1 (PROX-1) (Halsey, Worley, Curran, Charles, & Ehrhart, 2016).

3.2. Comparative molecular characteristics of canine hemangiosarcoma

The molecular characterization of canine HSA has primarily focused on gene expression analysis, array comparative genomic hybridizations studies to assess copy number aberrations (aCGH), and genome wide association studies (GWAS). The increased incidence of HSA in specific dog breeds (20% of Golden Retrievers will develop HSA) has made GWAS studies possible in dogs. In comparison, GWAS of human angiosarcoma would be difficult given its rare occurrence, GWAS analysis of HSA in Golden Retrievers identified 2 loci that predispose this breed to the development of both HSA and B-cell lymphoma. These loci do not involve changes in gene coding sequences, but appear to involve gene expression changes in pathways involved in T-cell mediated immune responses (Tonomura et al., 2015).

Gene expression analysis of canine HSA revealed elevated expression of 58 genes relative to hematomas. When gene expression in HSA was compared to other canine cancer types, significant elevation of *VEGFA, TIMP-1, FN-1, ADAM9, PDGFC, MMP14, TNFα*, and acid ceramidase was observed, implicating inflammatory and angiogenesis processes in the pathogenesis of cHSA (Tamburini et al., 2010). Recent data have suggested that HSA may derive from multipotent hematopoietic progenitors. Gene expression profiling of 24 samples identified groups characterized by angiogenesis, inflammation, and adipogenesis (Gorden et al., 2014). Similarly, gene expression profiling of human STS revealed elevation of genes associated with angiogenesis in human angiosarcomas, including: *TIE1, VEGFR2, SNRK, TIE2, VEGFR1, PECAM1, EPHA2, ANGPT2, EDNRB, PGF, FLI1*, and *VWF*. In contrast, KIT ligand, VEGFA and VEGFB were downregulated relative to other sarcomas. Activating mutations in VEGFR2 were identified in 20% of the samples (Antonescu et al., 2009).

Array CGH analysis of 75 visceral HSA tumors from 5 dog breeds revealed recurrent gains of canine chromosomes 13, 24, and 31 with loss of CFA16 (Thomas et al., 2014). Associated gains in genes within chromosomes 13 were PDGFRA (increased in 20% of cases), KIT (increased in 27% of cases), VEGFR2 (increased in 28% of cases), ANGPT1 (increased in 20% of cases). Loss of ANGPT2 and CDKN2AIP on CFA16 were observed in 20% of cases. Gain of ANGPT4 was observed in 31% of cases. Gain of VEGFA on CFA12 was measured in 29% of cases. Loss of CDKN2A/B was found in 28% of cases. Other significant oncogenes and tumor suppressors with observed variations in <20% of cases included: PDGFRB, TP53, MYC, and PTEN. Genomic Identification of Significant Targets in Cancer (GISTIC) (www.broadinstitute.org/cancer/cga/gistic) was used to discriminate cancer drivers from passenger alterations and identified 3 primary peaks: CFA 12 flanking VEGFA, CFA11 loss of the region containing *MTAP* and *CDKN2A/B*, and a gain in 21% of cases on CFA5 containing the *SKI* gene. Negligible correlation was observed with human angiosarcoma aCGH, which revealed *MYC* amplifications in a majority of secondary angiosarcomas and approximately half of the primary angiosarcomas (Italiano et al., 2012). Recent evaluation of 10 primary human cardiac angiosarcomas identified trisomy of chromosomes 4 (4 cases), 8 (providing an additional copy of *MYC* in 8 cases), 11 (3 cases), 17 (6 cases), and 20 (3 cases), as well as homozygous deletion of CDKN2 in 3 cases (Leduc, Jenkins, Sukov, Rustin, & Maleszewski, 2017).

Recent whole exome sequence (WES) analysis of formalin fixed paraffin embedded HSA and matched normal samples from 20 dogs of various breeds was analyzed to identifying

candidate driver mutations (Wang et al., 2017). Overall mutational burden for these samples fell in the low range of human tumors with 0.1–2.1 mutations per megabase. This study identified mutations predicted to have significant functional consequences in *PIK3CA*, (9 cases), *TP53* (7 cases), *PTEN* (2 cases), and a *PLCG1* mutation in one case. The *PIK3CA*, *PTEN* and PLCG1 were mutually exclusive mutations, and 8 of the 9 *PIK3CA* cases carried mutations at amino acid 1047 with six of the cases bearing the known human H1047R activating mutation, 2 cases with an H1047L mutation and the ninth case having a D350G gain of function mutation that blocks interaction of PIK3CA with the p85 negative regulator. Other mutations impacting the PI3 kinase pathway included inactivating mutations in PTEN and FOXO3. The phospholipase C gamma (PLCG1) mutation is homologous to an activating mutation in the human gene. Similar to human cancers, the seven *TP53* mutations were inactivating mutations localized to the DNA-binding domain. Eight of the cases lacked obvious driver mutations. These data suggest that activation of the PI3 kinase pathway is an important driver for canine HSA. Activating mutations in *PLCG1* have been observed in approximately 9% (3 of 34) of human angiosarcomas, a *PIK3CA* mutation was observed in 1case among a total of 39 tested (Behjati et al., 2014). The most prevalent gene mutations were inactivating mutations in *PTPRB*, a tyrosine phosphatase specific to vascular endothelium and associated with angiogenesis, observed in 26% (10/39) of the human angiosarcomas studied. The *PTPRB* mutations were identified in tumors with either *MYC* amplifications and/or known to be radiation associated secondary angiosarcomas. All three *PLCG1* mutations were found in tumors with *PTPRB* mutations.

MicroRNA (miRNA) Analysis by small RNA-seq of cHSA samples compared to nodular hyperplasia and normal spleen revealed 4 miRNas that were significantly differentially expressed (Grimes et al., 2016). Mir-126 and mir-452 were overexpressed with relative fold change increases of 3.38 and 13-fold respectively. Mir-150 and MiR-203 were decreased in expression by 2.8 and 2.56-fold. Mir-126, mir-150 and mir-203 have previously been associated with angiogenesis. Mir-126 appears to act to increase expression of VEGF by targeting the regulatory PI3K regulatory subunit 2.

Elevated expression of the KIT (CD117) receptor tyrosine kinase has been utilized to distinguish hemangiomas from HSA (Chen, Liao, Hsu, & Chang, 2016; Fosmire et al., 2004). In addition, alternative splicing of *c-kit* in canine HSA leads to an increase in the expression of a KIT isoform with a 12 nucleotide deletion in exon 9 that results in a GNSK deletion in the juxtamembrane region of the extracellular domain. The functional impact of this isoform is unclear, although loss of a similar GNNK motif in mice and humans has been associated with increased receptor activation and downstream signaling (Caruana, Cambareri, & Ashman, 1999; Voytyuk et al., 2003)

4. Fibrosarcoma

4.1. Comparative pathology of canine fibrosarcoma

Canine FSA is one of the histological subtypes included in the prognostic grading scheme used for the heterogeneous group of cutaneous and subcutaneous STS in dogs (Dennis et al., 2011; McChesney, Withrow, Gillette, Powers, & Dewhirst, 1989). At present, the clinical importance of distinguishing between FSA and other histologic subtypes of canine STS is

unknown. While several retrospective studies have suggested that FSA may carry a worse prognosis compared to other STS types, these studies lack proper survival analyses and adequate case numbers to draw significant conclusions on the prognostic significance of histologic type (Bostock & Dye, 1980; Kuntz et al., 1997). Nonetheless, improvement in the conduct of prognostic studies in veterinary oncology and the potential identification of prognostic differences between STS types requires increased accuracy in pathological diagnosis. Often canine FSA can be presumptively diagnosed based solely on its distinct histomorphological features. These include spindle shaped cells arranged in streaming, interwoven bundles which form a characteristic “herringbone” pattern in a background of dense collagenous stroma (Dennis et al., 2011). However, in less well-differentiated tumors, which lack resemblance to normal fibrous tissue, differentiation of FSA from other canine STS types can be difficult and requires the use of advanced molecular diagnostics.

Prior IHC studies have suggested S100 to be a marker of canine peripheral nerve sheath tumors (PNST) based on their presumed Schwann cell origin; however, additional studies have demonstrated expression of this protein in canine FSA, confounding the utility of this marker in separating these entities (Choi & Kusewitt, 2003; Gaitero, Anor, Fondevila, & Pumarola, 2008; Klopfleisch, Meyer, Lenze, Hummel, & Gruber, 2013; LaRock & Ginn, 1997). Further complicating this task, expression for α-SMA, a suggested marker for the identification of canine perivascular wall tumors (PWT; formerly termed hemangiopericytoma), another STS with similar histomorphological features, has also been reported in some canine FSA (Klopfleisch et al., 2013; Perez et al., 1996; Suzuki, Uchida, & Nakayama, 2014). PGP9.5 has been described as a marker for human PNSTs, yet unfortunately, overlapping expression of this protein is reported between canine PNST and FSA (Meyer & Klopfleisch, 2014). However, results from more recent studies evaluating differences in gene expression between canine FSA and PNST have shown promise in differentiating these two tumor types (Klopfleisch et al., 2013; Meyer & Klopfleisch, 2014). Specifically, RT-PCR analysis of GLI1 and GLEC3B gene expression demonstrated relatively high sensitivity and specificity for differentiating canine PNSTs from FSA (Meyer & Klopfleisch, 2014). In humans, certain molecular aberrations appear specific for distinct subtypes of FSA, including translocations between chromosomes 7 and 16 in low-grade fibromyxoid sarcoma and sclerosing epithelioid sarcoma, yet there is only a single report describing cytogenetic abnormalities in canine FSA (Aguirre-Hernandez et al., 2009; Folpe, 2014). Rearrangements of chromosome 11, including loss of heterozygosity in the CDKN2B-CDKN2A tumor suppressor gene cluster region was reported in two Labrador retrievers with poorly differentiated FSA (Aguirre-Hernandez et al., 2009).

4.2. Comparative molecular characteristics of canine fibrosarcoma

Limited molecular analysis of canine FSA has been conducted. Cytogenetic analysis of 2 canine FSA using chromosomal paints identified loss of CFA11q and trisomy of CFA30 in one tumor with translocations in chromosomes 4, 11, 27, and 30 in the other tumor (Sargan et al., 2005). Expanding on this study, the investigators identified multiple rearrangements of CFA11 with loss of heterozygosity in the region of the CDKN2A/B genes and the apparent variability of some exons of these genes (Aguirre-Hernandez et al., 2009). Differential gene expression has also been used to discriminate canine FSA and PNST (Klopfleisch et al.,

2013). A limited analysis of TP53 in 4 canine FSA samples showed no mutations (Nasir, Rutteman, Reid, Schulze, & Argyle, 2001).

The most important histo-pathologically recognized sub-type of canine FSA is a unique tumor occurring in the oral cavity of dogs, termed histologically low-grade, yet biologically high-grade FSA. These tumors typically arise in the maxilla and mandible, and the Golden Retriever appears to be a pre-disposed breed. As the name implies, histologically low-grade, biologically high-grade canine FSA are characterized by a strikingly bland histological appearance; however, these tumors display aggressive and locally infiltrative behavior, with a propensity for metastasis similar to other histologically high grade STS (Ciekot et al., 1994). Human aggressive fibromatosis or desmoid tumors are considered pathologically similar to this sub-type of canine FSA (Caspari et al., 1995; Ciekot et al., 1994; Couture et al., 2000; Miyaki et al., 1993) and are often observed in patients with familial adenomatous polyposis (APC) (Caspari et al., 1995; Couture et al., 2000; Miyaki et al., 1993). Familial APC patients with these tumors often carry mutations in the 3′ portion of the APC gene that alter its interaction with β-catenin (Caspari et al., 1995; Couture et al., 2000). Sporadic cases of desmoid tumors frequently carry mutations in codons 41 and 45 of β-catenin which results in increased stability (Jilong, Jian, Xiaoyan, Xiaoqiu, & Xiongzeng, 2007; Shitoh et al., 1999; Tejpar et al., 1999). In fact, recent whole exome sequencing has established that these tumors carry near universal disruption of the WNT/ β-catenin pathway (Crago et al., 2015). To date, there has been no molecular analysis of this histological subtype of oral canine FSA to determine if similar dysregulation of the WNT/β-catenin pathway exists.

While no comparable tumor has been described in dogs, congenital FSA in children are associated with *ETV6-NTRK3* (TEL-TRKC) gene fusions discovered by breakpoint analysis of the t(12;15)(p13;q25) translocation. These gene fusions are considered diagnostic for this tumor type (Lannon & Sorensen, 2005). *ETV6-NTRK3* receptor tyrosine kinase fusion protein activates downstream signaling cascades in the Ras-MAP kinase and PI3 kinase pathways and other *NTRK* gene family and *BRAF* fusions have been identified in similar pediatric spindle cell sarcomas (Church et al., 2017; Davis et al., 2017; Kao et al., 2018). In adult FSA, approximately 95% of human low-grade fibromyxoid sarcomas carry a FUS-CREB3L2 fusion gene, with the remainder typically having *FUS-CREB3L1* or *EWSR1-CREB3L1* fusions. *EWSR1-CREB3L1* fusions are also prevalent in the highly aggressive sclerosing epithelioid fibrosarcomas (SEF) (Arbajian et al., 2017). While these gene fusions are considered the drivers of both tumors, differences in malignancy may be due to microdeletions in the *DMD* gene encoding the dystrophin protein which may serve as a tumor suppressor in myogenic sarcomas (Wang et al., 2014). Observed chromosomal rearrangements in canine FSA suggest that fusion events may also drive these tumors in the dog (Aguirre-Hernandez et al., 2009).

5. Peripheral nerve sheath tumors

5.1. Comparative pathology of canine peripheral nerve sheath tumors

Similar to FSA, canine malignant PNST are also lumped into the heterogeneous and widely-inclusive diagnostic and prognostic grouping for all canine cutaneous and subcutaneous STS. A third type of STS also included within this grouping is canine hemangiopericytoma

(Dennis et al., 2011). Hemangiopericytoma in dogs was originally described as a sarcoma of pericyte origin based on some similarities in histological features to human hemangiopericytoma (Goldschmidt & Hendrick, 2008). Historically, canine hemangiopericytoma were considered to be distinguished from PNST based on the presence of distinctive perivascular whirling in the former, which is less prominent and typically surrounding sclerotic collagen, as opposed to capillaries, in PNST (Gaitero et al., 2008) (Fig. 2B). Despite these aforementioned descriptive characteristics, similar to canine FSA, considerable overlap in histomorphological and IHC features still can exist between canine hemangiopericytoma and PNST (Avallone et al., 2007; Chijiwa et al., 2004; Goldschmidt & Hendrick, 2008; Suzuki et al., 2014). Furthermore, even within the grouping of canine hemangiopericytoma, discordant IHC results for expression of S100, CD34, and SMA have been reported, suggesting canine hemangiopericytoma may be a non-specific diagnostic category encompassing neoplasms of several distinct histologies (Avallone et al., 2007; Mazzei, Millanta, Citi, Lorenzi, & Poli, 2002; Perez et al., 1996; Suzuki et al., 2014).

Consistent with these observations, IHC and ultrastructural studies over the last decade have led to a reclassification of canine hemangiopericytoma into a broad spectrum of STS derived from nonendothelial mural cells of blood vessels, termed canine peri-vascular wall tumors (PWTs) (Avallone et al., 2007; Palmieri et al., 2013). This novel classification scheme in dogs parallels prior pathological studies for human perivascular wall tumors, and similar to humans, the following subtypes have been described: myopericytoma, angioleiomyoma/sarcoma, hemangiopericytoma, and angiofibroma (Avallone et al., 2007; Palmieri et al., 2013). An IHC panel consisting of antibodies to heavy-caldesmon, smoothelin, myosin, calponin, and CMG-3G5 is suggested for the differentiation of subtypes of canine PWTs (Avallone et al., 2007). However, the utility of this differentiation for routine diagnostic and prognostic purposes in veterinary oncology is irrational, as a panel of this many markers is cost-prohibitive, and furthermore, these markers are only reported to work on fresh frozen tissue. As such, prognostic studies in veterinary oncology have treated canine PWTs as a single entity, and suggest that tumor size, depth, completeness of margins, and location (extremities) are associated with clinical outcome (Avallone et al., 2014; Stefanello, Avallone, Ferrari, Roccabianca, & Boracchi, 2011). Continued improvement in our understanding of the biology of canine STS and the impact of histologic type on prognosis requires the ability to routinely distinguish between tumor types in this grouping, especially PWTs and PNST. Along these lines, recent work by Suzuki et al. suggest that expression of nerve growth factor receptor (NGFR) and the transcription factor Olig2 are the most useful markers to distinguish between these two tumor types (Suzuki et al., 2014).

5.2. Comparative molecular characteristics of canine peripheral nerve sheath tumors

Genomic data associated with canine PNST tumors is sparse but analysis of mutations in TP53 have shown that 3 of 6 primary PNST tumors had mutations and that amplification of MDM2 was observed in 3 of the 7 (Nasir et al., 2001). It should be noted that a lung metastasis from one of the canine PNST primary tumors was also analyzed for TP53 mutation and showed the same $TP53^{R261H}$ mutation observed in the primary. Hotspot analysis for the canine homolog of the BRAFV600E mutation identified this activating mutation in 2 of 9 PNST (22%) (Mochizuki, Kennedy, Shapiro, & Breen, 2015).

Cytogenetic analysis of a canine PNST (neurofibroma) identified trisomy in CFA2, a derivative CFA13, and centric fusions of CFA10/35 and 24/31 (Mayr, Wagner, Schleger, & Reifinger, 1990). In comparison, many human malignant PNSTs are associated with neurofibromatosis type 1 (NF1) and this syndrome is associated with a 10–15% lifetime risk for the development of PNST. Recent examination of the genomic landscape in mPNST identified recurrent mutations in NF1 87.5%, polycomb repressor complex proteins (SUZ12 and EED are thought to activate RAS signaling 58%), TP53 (40.3%), and CDKN2A (75%). Additionally, non-recurrent mutations in a number of genes that were expected to be both pathogenic and RAS pathway activating were identified. (Brohl, Kahen, Yoder, Teer, & Reed, 2017) Overall, this suggests a profile of activating mutations in the RAS signaling pathway in conjunction with loss of regulation through P53.

6. Histiocytic sarcoma

6.1. Comparative pathology of canine histiocytic sarcoma

Canine HS encompasses a diverse group of proliferative diseases of both dendritic cell and macrophage origin and represents the malignant counterpart of histiocytic proliferative disorders of the dog (Moore, 2014). Grossly, these tumors can present as solitary or multiple, firm, white, nodular masses involving only a single tissue or organ, termed localized HS (Affolter & Moore, 2002). Common primary sites for localized HS often include the lung, lymph node, spleen, bone marrow, central nervous system, skin/subcutis, and periarticular tissues of the limbs (Moore, 2014). When these tumors spread beyond local draining lymph nodes to involve multiple distant organs, frequently including the liver and lungs, the disease is termed disseminated HS, the contemporary name for what was originally reported as malignant histiocytosis in Bernese Mountain Dogs (Moore, 2014; Moore & Rosin, 1986).

Canine HS is thought to arise from interstitial dendritic cells, based on its widespread tissue and multi-organ distribution, and a supporting immunophenotypic profile defined as CD11c/CD18+, MHCII+, CD1a+, and CD11d− (Affolter & Moore, 2002; Moore, 2014). Histologically, HS can display a varied appearance, ranging from sheets of large, pleomorphic round cells with marked anisocytosis and anisokaryosis, and frequent karyomegalic and multi-nucleated giant cells, to dense proliferations of spindle-shaped cells arranged in interlacing streams and bundles (Fig. 2C). In cases of the latter, these tumors are often morphologically indistinguishable from other canine sarcomas, and require demonstration of positive CD18 immunolabeling for diagnosis.

As previously mentioned, a distinct and highly aggressive subtype of HS, termed hemophagocytic HS, also exists in dogs (Moore et al., 2006). Grossly visible lesions of hemophagocytic HS are most prevalent in the spleen and liver, with splenic lesions often associated with infarcts (Moore et al., 2006). On further microscopic examination additional organ involvement most frequently including the bone marrow and lung is demonstrated (Moore et al., 2006). Immunophenotypic studies have demonstrated this tumor to be composed of a population of CD11d+ splenic and bone-marrow macrophages. Histologically, neoplastic cells diffusely expand and replace the splenic red pulp and often efface adjacent white pulp structures, while spread to the liver and lung is often

characterized by inconspicuous to extensive vascular invasion and colonization of hepatic sinusoids and pulmonary vasculature, with or without spread into the adjacent hepatic parenchyma and alveolar lumina, respectively (Moore, 2014; Moore et al., 2006).

Lastly, canine cutaneous histiocytoma is the benign form of histiocytic sarcoma in the dog, and represents a cutaneous neoplasm arising from histiocytes of Langerhan's cell origin (Moore, Schrenzel, Affolter, Olivry, & Naydan, 1996). Given the recent success of immunotherapy in the treatment of certain human cancers, an interesting characteristic feature of these benign neoplasms is the presence of a progressively increasing CD8+ T cell infiltrate, which are arranged in diffuse infiltrates as well dense nodular aggregates along the deep margin of the mass, and are associated with tumor cell necrosis and spontaneous regression frequently observed for these masses (Cockerell & Slauson, 1979; Moore et al., 1996). Comparatively, these tumors can rarely occur as multiple nodules to coalescing masses with extensive cutaneous involvement, and frequent involvement of regional lymph nodes and lungs, with potential for metastasis to a variety of other organs (Moore et al., 1996; Nagata, Hirata, Ishida, Hirata, & Nanko, 2000). This rare spectrum of disease is termed canine cutaneous Langerhan's cell histiocytosis (LCH), and Shar-pei dogs are reported to be an over-represented breed (Moore, 2014). Clinically distinct from solitary benign histiocytoma, these tumors share similarities with the single and multi-organ forms of human cutaneous Langerhan's cell histiocytosis (Moore, 2014; Moore et al., 1996).

6.2. Comparative molecular characteristics of canine histiocytic sarcoma

Histiocytic sarcomas occur in 15–25% of Bernese Mountain Dogs. To explore the factors that contribute to this increased risk, GWAS were conducted in groups of North American and European Bernese Mountain Dogs (Shearin et al., 2012). A single locus on CFA11 was identified in the North American cohort, while sites in both CFA11 and 14 were identified in the European group. The specific SNP conferring the increased risk for HS in CFA11 was not identified, however high density mapping of associated SNPs localized the case associated haplotype to the *CDKN2A/B* and *MTAP* genes. The presence of this haplotype was further correlated with increased expression of CDKN2A/B, but not MTAP.

Array CGH analysis of Bernese Mountain Dogs and Flat-coated retrievers identified recurrent losses (50–86%) in CFA 2, CFA 11, CFA 16, CFA 22 and CFA 31 (Hedan et al., 2011). Loss of a region in CFA16 was observed in 86% of tumors. Genes present in this region include *CDKN2A* interacting protein (*CDKN2AIP*), FAT tumor suppressor homolog1 (*FAT1*), Tumor suppressor candidate 3 (*TUSC3*), Mitochondrial Tumor Suppressor gene 1 (*MTUS1*) and pericentriolar material-1 (*PCM1*). Other recurrent losses in HCYs included the regions containing *CDKN2* (CFA11), *RB1* (CFA22), and *PTEN* (CFA26). A recent study identified *TP53* mutations in 12 of 26 dogs with 10 of those samples carrying a 2 bp insertion in exon 5 resulting in a stop codon (Asada et al., 2017). Recent studies in human HS have identified activating mutations in BRAF as the most frequent potential driving mutation in this rare human cancer (Go et al., 2014; Liu et al., 2016). Hot spot analysis for the activating V600E canine homolog did not identify a similar trend in 20 canine HS (Mochizuki et al., 2015). Next generation sequencing of human HS has also identified mutations in *KRAS, PTEN, PIK3CA, ASXL1, KIT, TP53* and *PTPN11* (Liu et al., 2016;

Wang, Li, Chen, & Liu, 2012). A *PTPN11* activating mutation E76K has been identified through analysis of a panel of 53 canine HS including tumors from 30 Bernese Mountain Dogs, 13 Golden Retrievers, and 10 dogs of other breeds (Thaiwong, Sirivisoot, Takada, Yuzbasiyan-Gurkan, & Kiupel, 2017). This activating mutation was identified in 11 of the Bernese Mountain Dogs (37%), but only 2 of the other samples (9%). This *PTPN11* gain of function mutation in the SHP2 non-receptor protein tyrosine phosphatase results in activation of the RAS signaling pathway and has been previously associated with various childhood leukemias, including juvenile myelomonocytic leukemia, B cell acute lymphoblastic leukemia, and acute myeloid leukemia with sporadic observation in adult solid tumors. Interestingly, a recent study associated CDKN2A/B deletion and/or mutations of MAP2K1 and NRAS with the aggressive behavior of Langerhans cell tumors implicating a CDKN2A/B with RAS/MAP kinase profile in aggressive histiocytic neoplasms (Xerri et al., 2017) similar to the observed alterations in CDKN2A/B and RAS pathway activation profiles in Bernese Mountain Dogs.

Gene expression profiling has been conducted to identify factors contributing to the development of HS in flat-coated retrievers (Boerkamp et al., 2013, 2014). RT-qPCR was used to confirm that *PPBP* (pro-platelet basic protein chemokine ligand 7), SpiC transcription factor, *VCAM1* (vascular cell adhesion molecule), *ENPEP* (Glutamyl aminopeptidase) and *ITGAD* (Integrin alpha D) were downregulated and *GTSF1* (gametocyte specific factor 1), *LUM* (lumican), Thy1 cell surface antigen and Col3a1 (Collagen Type III, alpha 1) were upregulated compared to normal spleen. Pathway analysis implicated pathways involved in DNA repair and replication including the P53 pathway in tumor development. Downregulation of receptor for glycation end products (RAGE) and high mobility box1 protein (HMGB1), factors contributing to the differentiation of dendritic cells has also been observed in HS (Sterenczak et al., 2011), as has upregulation of the antiapoptotic gene, survivin (Yamazaki, Takagi, Hoshino, Hosoya, & Okumura, 2013).

7. Osteosarcoma

7.1. Comparative pathology of canine osteosarcoma

Canine OSA shares remarkable clinical, biological, and histological similarities to human OSA (Withrow & Khanna, 2009). While OSA can occur in both the axial and appendicular skeleton, this review will primarily focus on appendicular OSA, as this is the most thoroughly documented and studied form of the disease in dogs, with appendicular OSA occurring ~3–4 times more often than axial OSA (Wolke & Nielsen, 1966). Appendicular OSA is a tumor of malignant osteoblasts which arises in the medullary cavity of the metaphyseal portion of long bones (Thompson & Pool, 2008) (Misdorp & Hart, 1979). The gross appearance of these tumors mimics the characteristic radiographic findings, and typically includes regions of trabecular and cortical bone lysis and destruction, with replacement and filling of the medullary cavity with variably firm, white to pale tan neoplastic tissue, as well as extensive production of endosteal and periosteal reactive bone (Thompson & Pool, 2008). Definitive histological diagnosis of OSA requires observation of osteoid production by malignant osteoblast cells; however, matrix production by these

neoplastic mesenchymal cells can be quite variable and range from abundant osteoid to a predominance of cartilage or collagen, which dictates sub-classification of these tumors.

Histological sub-classification of OSA is performed according to a scheme put forth by the World Health Organization, and is similar to the system utilized for human OSA (Slayter, 1994; Unni, 1996). These subtypes include osteoblastic (ranging from non-productive to productive), chondroblastic, fibroblastic, telangiectatic, giant cell type, and poorly differentiated. Osteoblastic OSA is the most common subtype and as the name implies, neoplastic osteoblast cells produce varying amounts of osteoid matrix (Slayter, 1994; Thompson & Pool, 2008). Similarly, chondroblastic OSA is characterized by production of both osteoid and chondroid matrices (Slayter, 1994; Thompson & Pool, 2008). Fibroblastic OSA is composed of dense streaming bundles of spindle cells strongly resembling FSA. This subtype of OSA is associated with a more favorable prognosis in both humans in dogs (Misdorp & Hart, 1979; Thompson & Pool, 2008). In contrast, the telangiectatic subtype of OSA is reported to be associated with increased metastasis and a less favorable prognosis than all other subtypes of OSA in both humans and dogs (Hammer, Weeren, Weisbrode, & Padgett, 1995; Unni, 1996). Histologically, this tumor is characterized by lysis of normal bone and replacement with pleomorphic mesenchymal cells which line variably sized blood-filled cystic spaces (Thompson & Pool, 2008) (Fig. 2D). Overall, almost all canine OSA, regardless of subtype, are histologically high grade tumors, which is reflected in the fact that ~90% of dogs have micrometastases at time of diagnosis (Kirpensteijn, Kik, Rutteman, & Teske, 2002; Withrow & Khanna, 2009). Pathological grading of canine OSA has been evaluated in two separate studies (Kirpensteijn et al., 2002; Moore et al., 2007). However, similar to the experience in human OSA, these systems have not been universally adopted as their prognostic significance remains controversial (Kirpensteijn et al., 2002; Moore et al., 2007; Resnick, 2002). One study of 166 dogs suggested grade III tumors are associated with a worse overall prognosis, while a separate study of 303 dogs concluded that mitotic rate was the only pathological variable predictive of survival (Kirpensteijn et al., 2002; Moore et al., 2007). Lastly, histologic evidence of regional lymph node metastasis in canine OSA, although rare and observed in ~5% of cases, is associated with a significantly shorter median overall survival in these patients (Hillers, Dernell, Lafferty, Withrow, & Lana, 2005).

Pathological studies on the molecular aspects of canine OSA have provided some key insights into the tumors pathogenesis. A study evaluating p53 expression by IHC in a subset of 106 osteogenic tumors in dogs demonstrated that p53 was significantly over-expressed in appendicular OSA as compared to axial OSA and multi-lobular osteochondrosarcoma, suggesting over-expression is associated with more aggressive clinical behavior (Sagartz et al., 1996). Osteoclastogenesis through the RANK-RANKL axis, and liberation of tumor-promoting growth factors from bone matrix, has been shown to be a major regulator in the establishment of secondary (metastatic) tumors of bone (Endo-Munoz, Evdokiou, & Saunders, 2012; Mundy, 1997). Similarly, osteoclast activation has also been implicated in progression and metastasis of primary canine OSA (Akiyama et al., 2010; Avnet et al., 2008), and a study by Barger et al. has demonstrated RANKL expression in 23/33 primary OSA (Barger, Fan, de Lorimier, Sprandel, & O'Dell-Anderson, 2007). IHC evaluation of COX-2 expression in a series of 44 appendicular OSA suggested that dogs with strong COX-2 expression had significantly decreased overall survival (Mullins et al., 2004). Ezrin,

a protein which has been shown to mediate pro-survival signals for early metastatic OSA cells, has been demonstrated to be expressed in a large percentage of OSA (Khanna et al., 2004). In this study of 73 dogs, high ezrin immunolabeling was associated with a significantly shorter median disease-free interval as compared to dogs with low ezrin expression in their primary tumors (Khanna et al., 2004). Interestingly, increased micro-vessel density of primary OSA, as assessed by F8RA IHC, has been associated with early pulmonary metastasis, a finding which parallels observations in human OSA (Coomber, Denton, Sylvestre, & Kruth, 1998; Kaya et al., 2000).

7.2. Comparative molecular characteristics of canine osteosarcoma

There is an increased incidence of OSA among the large and giant dog breeds such as Scottish Deerhounds, Irish wolfhounds, Rottweilers, and greyhounds. GWAS analysis was used to identify inherited risk loci in three breeds: greyhounds, Rottweilers, and Irish wolfhounds. This study identified 33 different loci that could effectively account for 55–85% of the phenotype variance. The identified loci were different for each of the 3 breeds, however one of the greyhound risk haplotypes (chr11:44,390,633–44,406,002) was fixed in each of the other breeds. Sequencing and dense haplotyping was used to narrow the region in canine chromosome 11 to a SNP near *CDKN2A/B* which is hypothesized to regulate expression of one or more of these genes through PAX5 binding. This specific SNP was not consistently associated with OSA risk across all breeds suggesting that breed variations across this region may modulate the impact of this variant. The corresponding region containing *CDKN2A/B* on human chromosome 9 is deleted in 5–20% of human OSA cases. Furthermore, significant connectivity was identified between the other loci with a majority of the gene regions associated with bone, development, and differentiation. Similar to the *CDKN2A/B* locus, it was found that fixed regions within the genomes of several breeds with a high risk of OSA overlap with genes associated with bone and OSA development such as *RB1, FOS, RUNX2, CCNB1, COL11A2*, and *POSTN*. Both GWAS and low genomic variability regions were enriched for the regulatory pathways KIT, p53, PDGFR, MAP Kinase, and AP1, as well as mir-124 regulated genes. Finally, when GWAS pathways were compared to the DNA copy number aberrations (CNAs) present in two breeds they found that CNAs were very similar in the two breeds as well as having significant similarities with human aCGH, with significant gains in *MYC* and *RUNX2* and losses in *RB1* and *CDKN2A/B*. GISTIC analysis overlapped with GWAS analysis with loss of the region containing *CDKN2A/B*. Other human and canine somatic gene changes associated with the GWAS analysis were loss of *BLMH*, *ARHGAP22*, *ARID5B*, *RCBTB1*, *LHFP*, *AIFM2*, and *TSC22D1*. Similar human GWAS analysis in 941 patients and 3291 unaffected individuals identified only 2 loci: one in the glutamate receptor *GRM4* (Savage et al., 2013) and the other more recently localized to a lincRNA by the ENCODE project (Bilbao-Aldaiturriaga, Martin-Guerrero, & Garcia-Orad, 2015). This gene was not identified in the canine GWAS, but another glutamate receptor, *GRIK4*, was identified as a locus in greyhounds. GWAS analysis also identified a germline SNP in *NFIB* that is significantly associated with metastasis in human OSA (Mirabello et al., 2015).

Array comparative genomic hybridization (aCGH) has been used to assess copy number variations which contribute to pathogenesis in canine OSA (Angstadt et al., 2011; Angstadt,

Thayanithy, Subramanian, Modiano, & Breen, 2012; Thomas et al., 2009). The largest of these studies incorporated data from 123 dogs with OSA and identified recurrent aberrations in regions containing genes previously associated with human OSA, including *TSC2, RHOC, RUNX2*, *MYC, TUSC3* and *PTEN* (Angstadt et al., 2011). A subset of these samples was further analyzed with high-resolution microarrays to identify micro-aberrations common to human and canine OSA (Angstadt et al., 2012). Identified regions common to both species included previously identified genes, but also expanded to include gains in *ADAM15* and *CTC1* and loss of *MEN1*, *CDK7*, and *CDKN2A/B*. Like human OSA, canine OSA is characterized by a chaotic karyotype. Recent SNP array analysis of 45 human OSA patients identified amplifications of chromosome 6p (containing genes for *E2F3*, *CCND3*, *RUNX2*, *VEGFA*, *KLH31*, and *ZNF45* among others), 8q (*MYC*), and 12q (*CDK4*) (Smida et al., 2010). The region most commonly exhibiting loss of heterozygosity (LOH) was the *RB1* locus (43%), other regions exhibiting LOH were *CDKN2A* (11%), *EGFR* (13%), and *PTEN* (13%) (Smida et al., 2010). Array CGH analysis of 38 canine OSA revealed that the most common genetic deletion in these tumors was loss of the *PTEN* tumor suppressor locus (41%) (Thomas et al., 2009).

Mutations of the tumor suppressor p53 and retinoblastoma (RB1) gene have been commonly associated with the development of OSA in humans and dogs (Fenger, London, & Kisseberth, 2014; Kansara & Thomas, 2007; Mueller, Fuchs, & Kaser-Hotz, 2007). Point mutations in p53 have been identified in approximately 41% of canine OSA tumors (Kirpensteijn, Kik, Teske, & Rutteman, 2008). In comparison, recent sequence analysis of human OSA samples identified mutations in the p53 pathway in each of 20 tumor samples analyzed. Nineteen of these samples exhibited direct mutation of p53, with 55% of those samples exhibiting structural variants with breakpoints confined to the first intron (Chen et al., 2014). Thus, while large structural variations in p53 are prevalent in human OSA, approximately 74% of canine mutations are point mutations (Kirpensteijn et al., 2008). Next generation sequence (NGS) analysis of 123 human OSA identified 14 putative driver genes which are suggested to account for 87% of OSA cases including: *TP53, RB1, BRCA2, BAP1, RET, MUTYH, ATM, PTEN, WRN, RECQL4, ATRX, FANCA, NUMA1*, and *MDC1* (Kovac et al., 2015). These variants have also been identified in other NGS studies (Bousquet et al., 2016; Chen et al., 2014; Perry et al., 2014). Similar studies have yet to be reported for canine OSA. Elevated expression of truncated ΔNp63 in canine OSA is associated with cellular survival and metastasis (Cam et al., 2016). In human OSA, loss of heterozygosity in RB1 was identified in 60% of tumors with structural rearrangements in 30% of samples and point mutations in 10% (Kansara & Thomas, 2007). Recent studies have identified losses in RB1 protein in canine OSA cell lines (Levine & Fleischli, 2000), and aCGH analysis identified copy number loss of RB1 in only 29% of cases. Differences in the prevalence of *TP53* and *RB1* deletions and mutations between human and canine samples may be accounted for by genomic loss of *CDKN2A/B* which would impact both the p53 and RB1 signaling pathways (Thomas et al., 2009).

In contrast, some genetic aberrations appear to be specific to certain dog breeds. Loss of Wilms Tumor 1, *WT1*, was identified in 48% of Rottweilers, but was not lost in Golden Retrievers (Scott et al., 2011; Thomas et al., 2009). In addition, a novel germline mutation was identified in the MET receptor in Rottweilers (Liao, McMahon, & London, 2006) and

dysregulation of MET expression has been associated with both human and canine OSA (MacEwen et al., 2003; McCleese et al., 2013).

Gene array expression analysis has been conducted on both canine and human OSA samples. Work by Paoloni et al. found that gene expression signatures for canine and human OSA are more similar to each other than to normal tissues from the same species (M. Paoloni et al., 2009). Gene expression analysis comparing tumors taken from dogs with short and long disease-free interval or survival commonly identified enrichment for pathways regulating hedgehog signaling, WNT signaling and chemokine signaling (O'Donoghue et al., 2010; Selvarajah et al., 2009). Unbiased cluster analysis of gene expression profiles in early passage canine OSA cultures grouped the samples into a group with overexpression of genes involved in mitosis, chromosomal segregation, and mitotic spindle formation versus a group with functions associated with cell migration, adhesion, angiogenesis, proliferation, and inflammation. Samples in the first group also had a significantly shorter overall survival. Similar profiles were observed when these parameters were assessed in other canine and human datasets (Scott et al., 2011). Overexpression of *RB1* in canine OSA cells from the first group shifted their gene expression profiles to more closely resemble those of the second group through altered E2F signaling (Moriarity et al., 2015). In addition to these more global evaluations of gene expression, a variety of studies have linked activation of signaling pathways including receptor tyrosine kinase-MAP kinase, Hedgehog (O'Donoghue et al., 2010; Shahi, Holt, & Rebhun, 2014), Notch (Dailey et al., 2013), Wnt (de Sa Rodrigues, Holmes, Thompson, Newton, & Stein, 2017; Piskun & Stein, 2016; Stein, Holmes, Muthuswamy, Thompson, & Huelsmeyer, 2011), STAT3 (Fossey et al., 2009), TGFβ, cellular survival (Shoeneman et al., 2012), ezrin (Hong et al., 2011; Khanna et al., 2004) and MTOR (Gordon, Ye, & Kent, 2008) in the pathogenesis and metastasis of canine OSA. These studies have been summarized in a recent reviews of canine (Fenger et al., 2014) and human (Kansara, Teng, Smyth, & Thomas, 2014) OSA (Table 2).

Analysis of miRNA expression profiles identified significant downregulation of miRNAs localized to the 14q32 locus in human OSA. This region includes a number of miRNAs predicted to target c-Myc including miR-382, miR-369–3p, miR-544, and miR-134. This interaction was confirmed and overexpression of these miRNAs also resulted in the downregulation of the myc regulated mir-17–92 cluster as well as inducing apoptosis in the Saos2 OSA cell line (Thayanithy et al., 2012). Loss of these miRNAs was further correlated with increased metastasis in human patients and decreased expression of miR-134 and miR-544 was confirmed in dogs with shorter survival times (Sarver et al., 2013). Nanostring technology has also been used to identify miRNAs differentially expressed between normal osteoblasts and canine OSA (Fenger et al., 2016). This analysis identified 26 miRNAs overexpressed in canine OSA (validated miR-9, miR-126, miR-199b, and miR-451) and 44 miRNAs were downregulated (validated miR-29a). They further confirmed that miR-9 contributes to invasion and migration in OSA cells through the downstream regulation of gelsolin.

8. Clinical management and outcomes in canine sarcomas

8.1. Surgical treatment of canine sarcomas

As is the case in the vast majority of human sarcomas, surgery remains the mainstay of therapy for localized canine sarcomas. Wide-margin excision is recommended owing to extensive microscopic tissue infiltration and a significant likelihood of local recurrence following conservative/marginal resection, with high grade/undifferentiated tumors more likely to recur (Bray, Polton, McSporran, Bridges, & Whitbread, 2014; Hohenhaus et al., 2016; McSporran, 2009).

8.2. Radiation therapy of canine sarcoma

Postoperative radiation therapy (RT) appears to decrease the likelihood of local recurrence for incompletely resected STS (Demetriou, Brearley, Constantino-Casas, Addington, & Dobson, 2012; Forrest, Chun, Adams, Cooley, & Vail, 2000; Hohenhaus et al., 2016; McKnight, Mauldin, McEntee, Meleo, & Patnaik, 2000), as is the case in humans. Comparatively high total radiation doses appear to be necessary for adequate control, although total dose and fraction number are generally lower than typical human protocols. A total dose of 54–60 Gray delivered in 18–20 daily (M–F) 3-Gray fractions over 4 weeks is often prescribed. The differences in total dose and fractionation versus human sarcomas are largely due to the need for anesthesia or heavy sedation for each treatment, and the attendant cost considerations.

Radiation therapy has been evaluated as a primary treatment modality for unresectable canine STS. “Definitive” or “curative intent” RT protocols, using protocols similar to those described above, are associated with approximately 12 month median durations of tumor control (McChesney et al., 1989). Recent studies suggest a high likelihood of long-term tumor control following high dose per fraction, stereotactic radiation therapy (SRT) protocols in dogs with OSA, providing a useful nonsurgical limb-sparing option for dogs that are not candidates for limb amputation (Boston et al., 2017; Farese et al., 2004). Pathologic fracture following SRT is a relatively common sequel, occurring in approximately 30–50% of unselected cases, and concurrent surgical stabilization appears to be associated with an unacceptable incidence of postoperative osteomyelitis (Boston et al., 2017).

Coarsely fractionated or “palliative” RT protocols, generally consisting of weekly fractions of 6–8 Gray, are associated with response rates of approximately 50% in dogs with STS and HSA, with median survival times (MSTs) of 3–6 months (Hillers, Lana, Fuller, & LaRue, 2007; Lawrence, Forrest, Adams, Vail, & Thamm, 2008; Poirier, Bley, Roos, & Kaser-Hotz, 2006). Similar palliative RT protocols are associated with improvements in pain in approximately 70% of dogs with appendicular OSA, with median durations of pain control of 2–4 months (Green, Adams, & Forrest, 2002; Mueller et al., 2005; Ramirez et al., 1999).

8.3. Medical therapy of canine sarcomas

In canine sarcomas, medical therapy is generally reserved for postoperative treatment of tumors that are incompletely resected or those that are at high risk for metastasis (HSA,

OSA, HS, high grade/undifferentiated STS). The postoperative use of low dose continuous (metronomic) chemotherapy, consisting of continuously administered oral cyclophosphamide and the nonsteroidal anti-inflammatory drug piroxicam, appeared to be associated with a reduced recurrence risk on one uncontrolled retrospective study (Elmslie, Glawe, & Dow, 2008). In sarcomas at high risk for metastasis, conventional (maximum tolerated dose) chemotherapy is the treatment of choice. As in humans, platinum drugs and/or doxorubicin (DOX) form the mainstay of postoperative chemotherapy in dogs with OSA, with approximate tripling of MSTs versus surgery alone (~4 versus ~12 months) (Selmic, Burton, Thamm, Withrow, & Lana, 2014). Similar improvements in postsurgical MSTs are observed with the addition of DOX- and lomustine-based chemotherapy in dogs with HSA and HS, respectively (Ogilvie et al., 1996; Skorupski et al., 2009; Sorenmo, Jeglum, & Helfand, 1993; Wendelburg et al., 2015). The role of postoperative chemotherapy in dogs with “high risk” STS is unknown.

In dogs with gross primary or metastatic disease, medical therapy is associated with limited efficacy, with conventional agents as above associated with response rates of <50% and median response durations of 3 months or less in canine STS, HSA and HS (Alvarez, Hosoya, Lara-Garcia, Kisseberth, & Couto, 2013; Rassnick et al., 2010; Skorupski et al., 2007; Wiley, Rook, Clifford, Gregor, & Sorenmo, 2010). Conventional cytotoxic therapy is nearly invariably ineffective for measurable metastatic canine OSA.

9. Clinical/translational investigations in canine sarcoma

9.1. Translational studies in surgical therapy utilizing canine sarcomas

One of the most significant contributions of the canine model to the development of human sarcoma therapy concerns National Cancer Institute funded work by Withrow and colleagues in the 1980s to develop methods and procedures for cortical allografts for limb-sparing surgery for patients with bone sarcomas. Surgical protocols were co-developed by human and veterinary surgical oncologists and refined in scores of dogs with spontaneous OSA, primarily of the distal radius, and the effects of pre-operative chemotherapy and RT were assessed (LaRue et al., 1989; Thrall et al., 1990; Withrow et al., 1993). The observations and refinements developed in dogs led directly to the implementation of these techniques in human musculoskeletal surgical oncology (Withrow & Wilkins, 2010). Interesting observations were made between the development of postoperative chronic bacterial osteomyelitis and improved oncologic outcome in dogs (Lascelles et al., 2005), which were subsequently confirmed in at least one cohort of humans with OSA (Jeys, Grimer, Carter, Tillman, & Abudu, 2007). Further interrogation of this phenomenon in a syngeneic murine model implicated NK- and monocyte-mediated suppression of angiogenesis as a possible mechanism of action (Sottnik, U’Ren, Thamm, Withrow, & Dow, 2010).

9.2. Translational studies of radiation therapy and hyperthermia in canine sarcoma

A large body of literature describes pioneering NCI-funded work of Dewhirst and colleagues describing the effects of hyperthermia and hyperthermia/RT combinations on the tumor microenvironment in canine STS. Owing to their peripheral location and the ease with which invasive procedures such as probe placement and serial biopsy can be performed, significant

insights in to thermal dosimetry, changes in tumor perfusion, tumor oxygenation and imaging/genomic predictors of response have been identified (Chi et al., 2011; Gillette et al., 1992; Thrall et al., 2012; Thrall, Larue, Pruitt, Case, & Dewhirst, 2006). Strategies for spatially-targeted drug delivery via thermosensitive liposomes have also been investigated in canine STS (Hauck et al., 2006).

9.3. Translational studies of biologic therapies in canine sarcoma

A variety of biologic therapies have been evaluated in canine sarcomas. In early-phase, proof-of-concept studies, these include tumor-targeting facultative anaerobic bacteria, delivered either systemically or locally (Roberts et al., 2014; Thamm et al., 2005), inhaled interleukin-2 liposomes for pulmonary metastatic OSA (Khanna et al., 1997; Khanna, Hasz, Klausner, & Anderson, 1996), systemically or intralesionally delivered IL-2 encoding cationic liposome DNA complex gene therapy (Dow et al., 2005; Thamm et al., 2003), and systemic administration of a PEGylated human tumor necrosis factor-alpha (Thamm et al., 2010). In all of these studies, evidence of safety/tolerability was produced, and encouraging antitumor activity was observed in select sarcoma cases.

Extensive work by MacEwen and colleagues in the 1980s and 1990s with the nonspecific immunomodulatory drug liposome muramyl tripeptide phosphatidylethanolamine (L-MTP-PE) was performed in dogs with OSA and HSA. In addition to randomized, placebo controlled trials demonstrating significant delay/prevention of metastasis and prolongation of survival following surgery and chemotherapy with L-MTP-PE (Kurzman et al., 1995; Vail et al., 1995), bronchoalveolar lavage preformed prior to and after L-MTP-PE treatment demonstrated significant enhancement of pulmonary alveolar macrophage cytotoxicity and activation status (Kurzman, Shi, Vail, & MacEwen, 1999). This body of work provided significant proof of principle demonstrating prevention of metastasis in OSA, which led directly to the conduct of a randomized, placebo-controlled trial in human OSA (Meyers et al., 2005), and the subsequent regulatory approval of L-MTP-PE (mifamurtide, Mepact™) by the European Medicines Agency for the treatment of OSA (Mifamurtide: CGP 19835, CGP 19835A, L-MTP-PE, liposomal MTP-PE, MLV 19835A, MTP-PE, muramyltripeptide phosphatidylethanolamine, 2008).

9.4. Translational studies of targeted toxin therapy in canine sarcoma

Two recent studies have evaluated targeted drug delivery in canine sarcoma models. In one, the nanoconjugate of a bisphosphonate drug with DOX was evaluated in dogs with OSA, and shown to be associated with the ability to significantly dose-escalate DOX without adverse effects (Yin et al., 2016). In a second study, dogs with HSA were treated postoperatively with a combination of DOX followed by an EGFR- and urokinase-targeted *Pseudomonas* exotoxin, referred to as eBAT. In additional to excellent tolerability, there was the appearance of improved outcome when compared with historical patients treated with DOX-based chemotherapy alone (Borgatti et al., 2017).

9.5. Translational studies of antiangiogenic therapies in canine sarcoma

The ease with which serial biopsy can be performed has allowed several studies evaluating tumor-specific therapeutic delivery or changes in tumor microvessel density following anti-

angiogenic therapy in canine sarcomas. These include a xenogeneic VEGF protein vaccine with a novel adjuvant (Kamstock, Elmslie, Thamm, & Dow, 2007), and dose-finding pharmacodynamic studies of low dose continuous (metronomic) cyclophosphamide (Burton, Mitchell, Thamm, Dow, & Biller, 2011). Two recent surgical adjuvant trials have been performed evaluating the multitargeted kinase inhibitor toceranib phosphate (Palladia™, Zoetis), which has great structural and kinase-inhibitory similarity with the human kinase inhibitor subitinib. In both studies, the addition of toceranib to standard of care therapy did not improve progression free or overall survival time (Gardner et al., 2015; London et al., 2015). A final study evaluated the neovasculature-specific delivery of an RGD-targeted AAV-phage vector expressing tumor necrosis factor alpha in dogs with tumors, including STS. Tumor endothelium-specific gene expression was documented, and objective tumor regressions were observed in two dogs (M.C. Paoloni et al., 2009).

9.6. Translational studies of chemotherapy in canine sarcoma

Several studies have evaluated novel chemotherapy delivery methods, or mechanisms to modify or optimize chemosensitivity. A novel liposomal cisplatin, SPI-77 was evaluated in a randomized trial as postoperative therapy for OSA, and compared with carboplatin, the standard of care. Despite the ability to deliver 5 times more cisplatin when liposome encapsulated than the maximum tolerated dose of free cisplatin in dogs, there was no improvement in metastasis free or overall survival time when compared with carboplatin. The results of this study, as well as other factors, contributed to the decision to halt clinical development of SPI-77 (Vail et al., 2002). Studies evaluating pulmonary-delivered DOX or paclitaxel via inhalation demonstrated safety and objective antitumor activity in dogs with spontaneous lung neoplasia, primarily sarcomas (Hershey et al., 1999).

A randomized, placebo-controlled trial evaluated the postoperative efficacy of OncoLAR, a long-acting repositol octreotide, when combined with platinum-based chemotherapy, as postoperative therapy in dogs with OSA. No improvement in outcome was observed, possibly owing to an insufficient reduction in measured serum IGF-1 concentrations (Khanna et al., 2002).

Following encouraging retrospective data demonstrating the ability of gene express-based predictors of drug sensitivity, using an algorithm termed COXEN, to predict postoperative outcome in dogs with OSA treated with DOX or carboplatin (Fowles, Brown, Hess, Duval, & Gustafson, 2016; Gustafson, Fowles, Brown, & Theodorescu, 2015), we are currently accruing canine OSA patients to a prospective study evaluating this methodology to select choice of postoperative chemotherapy. Successful demonstration of a survival benefit following COXEN-based postoperative treatment assignment will provide critical proof of concept justifying these approaches in humans.

10. Conclusions and future directions for the use of canine sarcomas in cancer pharmacology and therapeutics

Spontaneous canine sarcomas are a diverse group of mesenchymal tumors, where most of the histotypes observed in humans are represented. The relatively common occurrence of

these tumors in dogs versus humans affords a unique opportunity for translational research in tumor histotypes that may be extremely challenging to study in humans (e.g. OSA, HSA, HS). Multiple studies evaluating surgery, RT, chemotherapy and targeted therapies have provided useful preliminary data in support of clinical development of future human therapeutics. To the extent that they have been characterized, multiple dysregulated molecular pathways appear to be conserved between dog and human sarcomas, suggesting the potential ability to evaluate novel pathway-targeted therapies in dogs with sarcomas as a model for the human disease.

Although molecular characterization of canine sarcomas is incomplete and lags behind what has been performed in humans, significant similarities between canine and human have been observed. To more thoroughly validate the model, future studies should more extensively characterize canine sarcomas at the molecular level, with special attention paid to accurate histological subtyping to facilitate comparison with the human analog.

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Abbreviations:

CSA	chondrasarcoma
FSA	fibrosarcom
GI	gastrointestinal tract
GIST	gastrointestinal stromal tumors
GWAS	genome wide association studies
HS	histiocytic sarcoma
HSA	hemangiosarcoma
IHC	immunohistochemistry
LIP	liposarcoma
LMY	leiomyosarcoma
LYA	lymphangiosarcoma
MYX	myxosarcoma
OSA	osteosarcoma
PNST	peripheral nerve sheath tumors
PWT	peri-vascular wall tumors

RMS	rhabdomyosarcoma
SCS	synovial cell sarcoma
STS	soft tissue sarcoma

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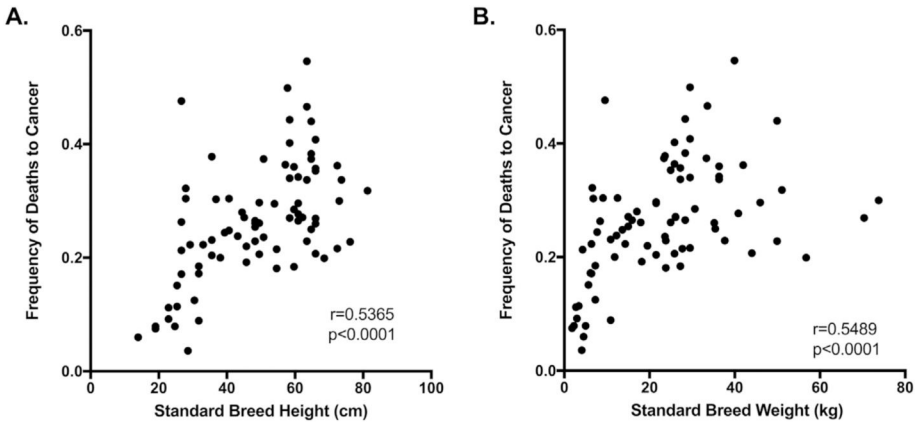

Fig. 1.

Correlation of Cancer Mortality to Standard Breed Height (A) and Weight (B) in North American Dogs. Frequency of death from cancer is from Fleming et al., 2011, and standard breed height and weight were compiled from breed information from the American Kennel Club (http://www.akc.org/dog-breeds). Correlation value (r) and significance (P) were calculated using a Spearman correlation test using GraphPad Prism v7.0a (GraphPad Software, Inc., La Jolla, CA).

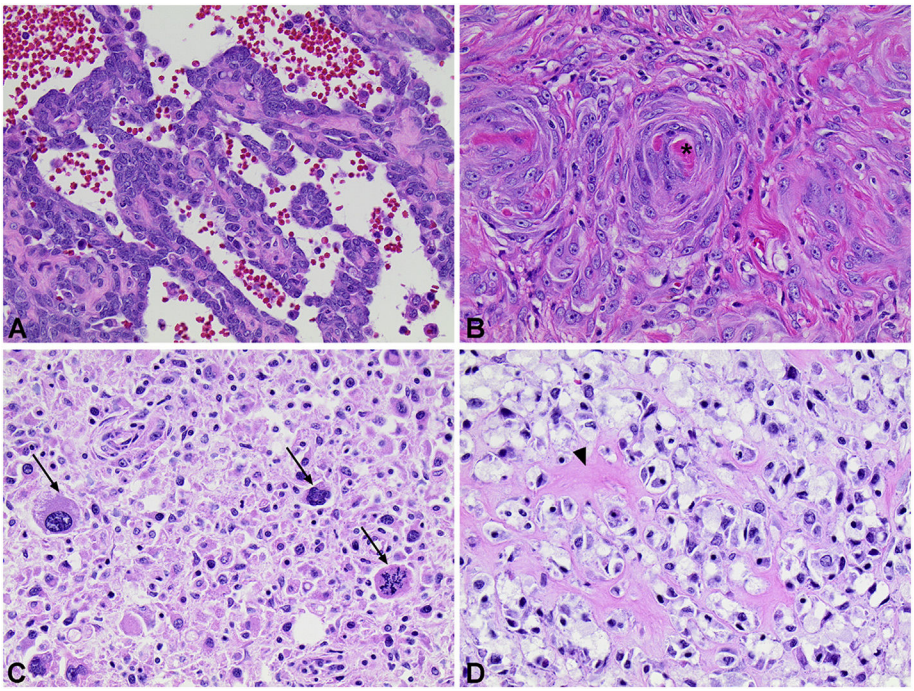

Fig. 2.

Histological features of common canine sarcomas. (A) Malignant peripheral nerve sheath tumor demonstrating characteristic concentric whirling of spindle cells around central sclerotic collagen (asterisk). (B) Osteoblastic osteosarcoma with polygonal neoplastic osteoblasts producing and embedded within eosinophilic neoplastic bone matrix (arrowhead). (C) Histiocytic sarcoma, characterized by marked cellular pleomorphism, with multi-nucleate and karyomegalic cells, and bizarre mitotic figures (arrows). (D) Splenic hemangiosarcoma. Neoplastic cells are forming irregular, anastomosing, blood-filled vascular spaces. 20x magnification, H&E staining.

Table 1

Primary anatomic location and estimated incidence of sarcomas in pet dogs in the United States.

Tumor type	Tissue location	% of dog cancers[a]	Estimated annual incidence in US[b]
Hemangiosarcoma	Spleen, heart, liver, subcutis, dermis	2.8%–5.0%	2156–10,600
Fibrosarcoma	Subcutis and oral cavity	3.0%–3.4%	2310–7208
Peripheral nerve sheath tumor	Subcutis	1.7%–5.6%	1309–11,872
Myxosarcoma	Subcutis	0.2%	154–424
Liposarcoma	Subcutis	0.2%–0.3%	154–636
Rhabdomyosarcoma	Tongue, larynx, heart, urinary bladder	0.1%	77–212
Leiomyosarcoma	GI tract, liver, vagina, subcutis	0.1%	77–212
Synovial cell sarcoma	Joints		
Lymphangiosarcoma	Subcutis, often trunk	0.1%	77–212
Histiocytic sarcoma	Joints, liver, spleen, lung, subcutis	4.0%	3080–8480
Osteosarcoma	Appendicular/axial skeleton (85%/15%)	0.9%–2.8%	693–5936
Chondrosarcoma	Nasal cavity, ribs, extremities	0.3%–0.4%	231–848

[a]Values are estimated based on survey and registry data from multiple databases (Bastianello, 1983; Dorn et al., 1968; Gruntzig et al., 2016; MacVean et al., 1978).

[b]Incidence is calculated based on an overall cancer incidence estimated at 99.3–272.1 per 100,00 dog years and a canine population in the United States of approximately 78 million. The range includes the variability in both the % of tumors represented by each tumor type as well as the incidence range (Merlo et al., 2008).

Table 2

Cancer gene census canine sarcomas.

Gene symbol[a]	Human tumor types	Human mutation types[b]	Alterations in canine sarcomas	Druggable target[c]
AKT1	Breast, colorectal, ovarian, NSCLC	Mis	CN loss 20% HSA	Yes
ASPSCR1	Alveolar soft part sarcoma	T	CN gain 29% OSA	
ATP2B3	Adrenal aldosterone producing adenoma	O	CN loss 20% HSA	
BRAF	Multiple tumor types	Mis, T, O	M — PNST	Yes
BRD4	Lethal midline carcinoma of young people	T	CN gain 20% HSA	Yes
CALR	MPN, MDS	F, Mis	CN gain 20% HSA	
CBFA2T3	AML	T	CN gain 20% HSA	
CCDC6	Papillary thyroid, CML, NSCLC	T	S — PNST	
CCND3	MM	T	CN gain 20% HSA	Yes
CDK6	ALL	T	CN gain 20% HSA	Yes
CDKN2A	Melanoma, multiple other tumor types	D, Mis, N, F, S	D, CN loss 28% HSA; CN loss 62% HSY; LOH FSA; GWAS OSA, HSY: CN loss OSA	Yes
DNAJB1	Fibrolamellar hepatocellular carcinoma	T	CN gain 20% HSA	
DNM2	T-ALL	F, N, S, Mis, O	M — HSA,CN gain 20% HSA	
DNMT3A	AML	Mis, F, N, S	Mis — PNST	Yes
EIF3E	Colorectal	T	CN gain 22% OSA	
ELK4	Prostate	T	CN loss 20% HSA	
ELL	AL	T	CN gain 20% HSA	
ERG	Ewing sarcoma, prostate, AML	T	CN gain 20% HSA	Yes
EXT1		Mis, N, F, S	CN gain 20% HSA	
EZH2	DLBCL	Mis	CN gain 20% HSA	Yes
FAT1	Oral squamous cell, chemorefractory CLL, head and neck, pancreatic acinar cell carcinoma	Mis, N, F, S	CN loss 20% HSA, CN loss 86% HSY	Yes
FGFR1	MPN, NHL, salivary adenoma	T	CN loss 20% HSA	Yes
FIP1L1	Idiopathic hypereosinophilic syndrome	T	CN gain 20% HSA, FS — PNST	Yes
FOXO3	Acute leukemia	T	Mis — HSA	
GNAS	Pituitary adenoma, pancreatic intra. Pap. mucinous neoplasm, fibrous dysplasia	Mis	CN gain 20% HSA, CN gains OSA	Yes
HOOK3	Papillary thyroid	T	CN loss 20% HSA, CN loss 16% OSA	

Gene symbol[a]	Human tumor types	Human mutation types[b]	Alterations in canine sarcomas	Druggable target[c]
HSP90AB1	NHL	T	CN gain 20% HSA	
JUN	Sarcoma	A	Mis — PNST	Yes
KAT6A	AML	T	CN loss 20% HSA	Yes
KCNJ5	Adrenal adenoma	Mis	Mis — PNST	
KDR	NSCLC, angiosarcoma	Mis	CN gain 28% HSA, CN gains OSA	Yes
KEAP1	NSCLC, breast carcinoma	Mis, N, F	CN gain 20% HSA	Yes
KIT	GIST, AML, TGCT, mastocytosis, mucosal melanoma	Mis, O	CN gain 27% HSA, CN gains OSA	Yes
KMT2C	Medulloblastoma	N	Deletion — PNST	Yes
LMNA	Spitzoid tumor	T	CN gain 20% HSA, Mis — PNST	
MDM2	Sarcoma, glioma, colorectal, other tumor types	A	CN gains OSA, PNST	Yes
MED12	Uterine leiomyoma, fibroadenoma, phyllodes tumor	Mis, S, O	Mis — leiomyomas	Yes
MLF1	AML	T	Mis — PNST	
MLLT3	ALL	T	CN loss 20% HSA	
MTOR	Multiple tumor types	Mis, N	CN gain 20% OSA	Yes
MYC	Burkitt lymphoma, amplified in other cancers, B-CLL	A, T	CN gain 17% HSA, CN gain 25% OSA	Yes
NCOR2	Prostate	Mis, F, N, O	CN gain 20% HSA	
NF1	Neurofibroma, glioma	D, Mis, N, F, S, O	Mis — PNST	Yes
NFATC2	Ewing sarcoma	T	CN gain 20% HSA	
NFIB	Adenoid cystic carcinoma, lipoma	T	CN gain 20% HSA	
NOTCH2	Marginal zone lymphoma, DLBCL, bladder	N, F, Mis	CN gain 20% HSA	Yes
NRG1	NSCLC	T	CN loss 20% HSA	
P2RY8	B-ALL, Down syndrome associated ALL	T	CN loss 20% HSA	
PAX7	Alveolar rhabdomyosarcoma	T	CN loss 20% HSA	
PCM1	Papillary thyroid, CML, MPN	T	CN loss 20% HSA, CN loss 18% OSA, CN loss 86% HSY	
PDGFRA	GIST, idiopathic hypereosinophilic syndrome, glioblastoma	Mis, O, T	CN gain 20% HSA	Yes
PIM1	NHL	T	Mis, N — PNST; CN gains OSA	Yes
PLCG1	Angiosarcoma	Mis	Mis — HSA, CN gain 20% HSA, CN gain 12% OSA	
PRKACA	Fibrolamellar hepatocellular carcinoma, cortisol secreting adrenal adenoma	T, Mis, N	CN gain 20% HSA, CN gains/losses OSA	
PTCH1	Skin basal cell, medulloblastoma	Mis, N, F, S	M — PNST	Yes
PTEN	Glioma, prostate, endometrial	D, Mis, N, F, S	Mis, N, D — HS, OSA; Mis, CN loss HSA b20% loss, CN loss 30% OSA, CN loss 40.7% HSY	Yes

Gene symbol[a]	Human tumor types	Human mutation types[b]	Alterations in canine sarcomas	Druggable target[c]
PTPN11	JMML, AML, MDS	Mis	Mis — HS	Yes
PTPRT	HNSCC, colorectal, gastric, lung cancer, melanoma	Mis, N	CN gain 20% HSA, CN gain 12% OSA	Yes
RB1	Retinoblastoma, sarcoma, breast, small cell lung carcinoma	D, Mis, N, F, S	CN loss 29% OSA, CN loss 56% HSY	Yes
RECQL4		N, F, S	CN gain 20% HSA	Yes
RNF213	ALCL	T	CN gain 29% OSA	
RSPO2	Colorectal	T	CN gain 22% OSA	
RUNX1	AML, pre B-ALL, T-ALL	T	CN gain 20% HSA	Yes
SALL4	Colorectal cancer, breast cancer, prostate cancer, glioblastoma, melanoma	Mis, F	CN gain 20% HSA	
SDC4	NSCLC	T	CN gain 20% HSA	
SLC45A3	Prostate	T	CN loss 20% HSA	
SND1	Pancreas acinar carcinoma	T	CN gain 20% HSA	
SRC	Colorectal cancer, endometrial carcinoma	Mis, N	CN gain 20% HSA	Yes
SS18L1	Synovial sarcoma	T	CN gain 20% HSA	
STK11	NSCLC, pancreatic	D, Mis, N, F, S	CN gain/loss 20% HSA, CN gain 15% OSA	Yes
TERT	Multiple tumor types	Promoter Mis	CN loss 20% HSA, CN gain 25% OSA	Yes
TFEB	Renal cell carcinoma (childhood epithelioid)	T	CN gain 20% HSA	
TNFRSF14	Follicular lymphoma	Mis, N, F	CN gain 20% HSA	Yes
TOP1	AML	T	CN gain 20% HSA, CN gain 12% OSA	Yes
TP53	Breast, colorectal, lung, sarcoma, adrenocortical, glioma, Spitzoid tumor, multiple other tumor types	Mis, N, F, T	Mis, N, F — OSA; CN loss 18% OSA; Mis, N — HSA, Mis, N — HS; CN gain 27% HS; Mis — MYX; Mis — LMS, Mis — PNST	Yes
TSC2	Pulmonary lymphangioleiomyomatosis (LAM), renal angiomyolipoma, HNSCC	D, Mis, N, F, S	CN gain 20% HSA, CN gain 16% OSA	Yes
U2AF1	CLL, MDS	Mis	CN gain 20% HSA	Yes
WHSC1L1	AML	T	CN loss 20% HSA	
WRN		Mis, N, F, S	CN loss 20% HSA	
ZFHX3	Endometrial, gastric, prostate	Mis, N	CN gain/loss 20% HSA, CN gain 15% OSA	

[a] Human mutation data from http://cancer.sanger.ac.uk/census.

[b] Mutation types: A — amplification, D — deletion, F — frameshift, Mis — missense, N — nonsense, O — other, S — Splice site, T — translocation.