

Canine Osteosarcoma: A Naturally Occurring Disease to Inform Pediatric Oncology

Joelle M. Fenger, Cheryl A. London, and William C. Kisseberth

Abstract

Osteosarcoma (OSA) is the most common form of malignant bone cancer in children and dogs, although the disease occurs in dogs approximately 10 times more frequently than in people. Multidrug chemotherapy and aggressive surgical techniques have improved survival; however, new therapies for OSA are critical, as little improvement in survival times has been achieved in either dogs or people over the past 15 years, even with significant efforts directed at the incorporation of novel therapeutic approaches. Both clinical and molecular evidence suggests that human and canine OSA share many key features, including tumor location, presence of microscopic metastatic disease at diagnosis, development of chemotherapy-resistant metastases, and altered expression/activation of several proteins (e.g. Met, ezrin, phosphatase and tensin homolog, signal transducer and activator of transcription 3), and p53 mutations, among others. Additionally, canine and pediatric OSA exhibit overlapping transcriptional profiles and shared DNA copy number aberrations, supporting the notion that these diseases are similar at the molecular level. This review will discuss the similarities between pediatric and canine OSA with regard to histology, biologic behavior, and molecular genetic alterations that indicate canine OSA is a relevant, spontaneous, large animal model of the pediatric disease and outline how the study of naturally occurring OSA in dogs will offer additional insights into the biology and future treatment of this disease in both children and dogs.

Joelle M. Fenger, DVM, Diplomate ACVIM (Oncology), is a graduate student in the Department of Veterinary Clinical Sciences at The Ohio State University College of Veterinary Medicine in Columbus, Ohio. Cheryl A. London, DVM, PhD, Diplomate ACVIM (Oncology), is the Shackelford Professor of Veterinary Medicine in the Department of Veterinary Biosciences at The Ohio State University College of Veterinary Medicine and Director of the Clinical Trials Office at The Ohio State University Veterinary Medical Center in Columbus, Ohio. William C. Kisseberth, DVM, PhD, Diplomate ACVIM (Oncology), is an associate professor in the Department of Veterinary Clinical Sciences at The Ohio State University College of Veterinary Medicine, and Director of the Biospecimen Repository at The Ohio State University Veterinary Medical Center in Columbus, Ohio.

Address correspondence and reprint requests to Dr. William C. Kisseberth, Department of Veterinary Clinical Sciences, The Ohio State University, 601 Vernon L. Tharp Street, Columbus, OH 43210 or email William.Kisseberth@cvm.osu.edu.

Key Words: comparative oncology; dog; naturally occurring cancer models; osteosarcoma

Introduction

The Dog as a Model for Cancer

Naturally occurring cancers in dogs have an intrinsic advantage as a model for human disease in that they mimic and represent biologically complex conditions in a way that is not possible using other animal models. Pet dogs are exposed to many of the same environmental factors as humans, as they share the same living environment. Similar environmental, nutrition, age, sex, and reproductive factors lead to tumor development and progression in human and canine cancers. Spontaneous cancers in pet dogs occur in the presence of an intact immune system and are characterized by tumor growth over long periods of time, interindividual and intra-tumoral heterogeneity, development of recurrent or resistant disease, and metastasis to relevant distant sites (Khanna et al. 2006). Importantly, the genetic structure of dog populations, that is, the presence of dog breeds, offers many advantages for genomic analysis. Breed creation has inadvertently selected for many “founder” mutations that are associated with specific traits and diseases; this translates into reduced disease and genetic heterogeneity. Because linkage-disequilibrium is up to 100-fold greater in dogs than humans, single breeds are powerful subjects for broad genetic mapping, whereas related breeds that share a trait are ideal subjects for fine mapping (Rowell et al. 2011). In addition, sequencing of the domestic dog genome and analysis of single nucleotide polymorphisms provide evidence of genetic diversity similar to that seen in human populations (Lindblad-Toh et al. 2005). There is now a growing body of evidence from cross-species genomic analyses that demonstrate significant similarities between genomic profiles in canine and human cancers, providing support for the notion that these diseases are similar on a molecular level (Paoloni et al. 2009; Richards et al. 2013). Importantly, pet dogs represent a large population size (>70 million in the United States), and their owners are highly motivated to seek out new treatment options for their pets, which provides a unique opportunity to sufficiently power clinical trials, including the assessment of new drugs (Khanna et al. 2006, Paoloni and

Khanna 2008). Given their large size, the evaluation of novel therapeutic approaches (drugs or devices) in pet dogs can answer important questions regarding relevant drug exposure that are often inadequately considered in mouse models. Furthermore, serial tumor biopsies and repeated collection of body fluid (serum, whole blood, urine) from dogs before, during, and after exposure to an investigational agent allows for evaluation of clinical and biologic end points, that is, pharmacokinetics and pharmacodynamics, that can be linked to drug exposure, surrogate imaging or circulating biomarkers, and therapeutic response in ways that are often difficult or unacceptable in human trials (Gordon et al. 2009).

The incorporation of dogs in cancer research is not a novel concept, and several organized cooperative efforts are now in place in the United States that are actively integrating pet dogs with cancer into the development path of new cancer drugs. The Veterinary Cancer Society and the Veterinary Cooperative Oncology Group have led efforts to encourage multicenter collaborative veterinary oncology studies. In addition, the Comparative Oncology Program of the National Cancer Institute at the National Institutes of Health has established the Comparative Oncology Trials Consortium to conduct rigorously controlled and focused preclinical trials of new cancer drugs intended to inform the design of human studies. Finally, the Canine Comparative Oncology and Genomics Consortium has established a canine cancer 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.

The Dog as a Spontaneous Model of Osteosarcoma

Osteosarcoma (OSA) is the most common primary malignancy of bone in both dogs and children. OSA in both species share many features, including the presence of microscopic tumor spread at diagnosis, similar responses to traditional treatment regimens such as surgery and chemotherapy, and dysregulation of several key molecular pathways (Table 1). Despite aggressive treatment, no improvement in survival times has been achieved in the past 15 years, with 30 to 40% of children and >90% of dogs still dying from disease.

To study OSA, several animal models have been developed to inform the human disease, including a variety of transgenic and xenograft rodent models (Mohseny et al. 2012; Sottnik et al. 2010; Walkley et al. 2008); however, these do not truly recapitulate the biology of OSA that occurs spontaneously in vivo, particularly with respect to primary tumor location. Mouse models of cancer have proven to be excellent tools for dissecting the biology of molecular pathways involved in cancer development and progression; however, they frequently do not adequately represent many of the features that define cancer in humans, including genomic instability and the heterogeneity of tumor cells within a complex microenvironment. Importantly, in

these genetically engineered mouse models, primary tumors occur most commonly in the flat bones, in contrast to the long bones in children and dogs (Walkley et al. 2008). Furthermore, conventional mouse models fail to recapitulate the complex biology of cancer recurrence and metastasis integral to outcomes in human patients.

The ability to rapidly advance therapeutics in pediatric OSA is limited by the low incidence of this disease in humans. In contrast, OSA is at least 10 times more prevalent in dogs. This provides a significantly larger patient population in which to evaluate new treatment strategies. Furthermore, the naturally shorter lifespan of dogs compared with humans, coupled with the short survival times achieved with current commonly used approaches, that is, combined surgery and adjuvant cytotoxic chemotherapy, accelerates the pace at which clinical trials in dogs can be conducted and allows for more rigorous evaluation before translation into new human trials.

Epidemiology and Etiology of OSA

Incidence and Risk Factors

OSA is the most common primary bone tumor in humans and dogs; however, the disease is significantly more common in dogs than in people. The estimated OSA incidence in dogs is at least 13.9/100,000 (Rowell et al. 2011) as opposed to an incidence of 1.02/100,000 in humans (across all ages) (Mirabello et al. 2009), classifying OSA in people as an orphan disease. OSA in both species has a bimodal age distribution; however, a key clinical difference is that the peak onset of the disease in humans is in adolescence (10- to 14-year-old age group) coinciding with the pubertal growth spurt (Ottaviani and Jaffe, 2009), whereas in dogs, OSA tends to occur in middle-aged to older dogs (median 7 years) after closure of the growth plates (Ehrhart et al. 2013). Historically, the incidence of OSA in dogs and humans has been considered to be higher in males than in females (Linabery and Ross 2008; Misdorp and Hart 1979; Spodnick et al. 1992); however, more recent data suggest an equal sex distribution in both species (Ehrhart et al. 2013; Ottaviani and Jaffe, 2009).

OSA most commonly occurs in the long bones of the appendicular skeleton near the metaphyseal growth plates. The first peak age of onset of OSA in humans occurs during the adolescent growth spurt, suggesting a close relationship between the rapid bone growth at the onset of puberty and tumor development (Longhi et al. 2005). The occurrence of OSA in anatomic sites of greater growth and in taller individuals suggests that growth factors play an important role in the pathogenesis of this disease. Canine OSA occurs in older dogs well after closure of the growth plates; however, increasing weight and tall shoulder height appear to be the most predictive factors for the development of this cancer in dogs (Ru et al. 1998). The association between canine OSA

Table 1 Comparison of Canine and Human Osteosarcoma Characteristics

Variable	Canine	Human
Incidence	>10,000/year	1,000/year
Age of onset	Middle-aged to older dogs Peak onset 7–9 years Second small peak at 18–24 months	Adolescent disease Peak onset 10–14 years
Race/breed	Large or giant breeds Increased inherited risk in Scottish Deerhounds, Rottweilers, greyhounds, Great Danes, Saint Bernards, Irish wolfhounds	None
Site	75% in the appendicular skeleton Metaphyseal region of long bones Distal radius > proximal humerus > distal femur	90% in the appendicular skeleton Metaphyseal region of long bones Distal femur > proximal tibia > Proximal humerus
Etiology	Generally unknown Ionizing radiation Bone infarcts Chronic osteomyelitis Metallic orthopedic implants Previous fracture/trauma	Generally unknown Ionizing radiation Bone infarcts Chronic osteomyelitis Paget's disease (>40 yrs of age)
Genetic and Molecular Alterations	See Table 2	See Table 2
Histopathology	95% high grade	85–95% high grade
Percentage clinically confined to the limb at presentation	85–90%	85–90%
Metastatic rate without chemotherapy	90% before 1 year	85–90% before 2 years
Metastatic sites	Lung > bone > soft tissues Regional lymph nodes 4.4%	Lung > bone > soft tissues Regional lymph nodes <10%
Prognostic factors	Appendicular/axial tumor location Proximal humeral location Metastasis at diagnosis Incorporation of chemotherapy Postoperative limb sparing infection Tumor size Serum alkaline phosphatase	Appendicular/axial tumor location Proximal humeral location Metastasis at diagnosis Incorporation of chemotherapy Postoperative limb sparing infection Tumor size Local tumor recurrence Percent tumor necrosis Age (>65 years of age)

Downloaded from https://academic.oup.com/ilarjournal/article/55/1/69/841918 by guest on 22 May 2023

and bone growth is further supported by the increased prevalence of the disease in large and giant breed dogs and by studies demonstrating the expression of the insulin-like growth factor-1 (IGF-1) receptor and ligand in canine OSA cells (MacEwen et al. 2004). Anatomically, the site of OSA development in children and dogs is strikingly similar, with

a predilection for the weight-bearing region of long bones. Approximately 75% of canine OSA occurs in the appendicular skeleton, with the distal radius and proximal humerus being the two most common locations (Knecht and Priester 1978). In human OSA, long bones are affected in up to 90% of cases, with the most common sites in the distal femur,

proximal tibia, and the proximal humerus (Dahlin and Unni 1986).

Environmental and Physical Factors

The etiology of OSA in both humans and dogs is generally unknown; however, it likely involves a complex interaction involving environmental and physical factors, genetic susceptibility, and acquired molecular aberrations. Experimental and therapeutic ionizing radiation is a well-documented etiologic factor known to induce OSA in dogs and humans. In the experimental setting, plutonium-induced OSA in beagle dogs mimics the anatomic distribution of OSA in humans exposed to plutonium, with the majority of lesions located in the axial skeleton (Miller et al. 2003). This anatomic distribution of OSA differs from the spontaneously occurring OSA in both species and appears to be related to bone volume and turnover. Secondary radiation-induced OSA comprises approximately 3% of all human OSA, with a prolonged latent interval between radiotherapy (RT) and diagnosis of sarcoma (mean 17 years, range 4–50 years) (Ottaviani and Jaffe 2009). In dogs, radiation-induced OSA is a similarly rare, late complication. It was reported to develop within the field of radiation in 3 of 87 (3.4%) dogs treated for soft tissue sarcomas (Gillette et al. 1990) and 3% of 57 dogs irradiated for acanthomatous epulis of the oral cavity (McEntee et al. 2004). Among humans with childhood cancers, those with Ewing sarcoma are at highest risk of subsequent OSA because of the high radiation doses (41–60 Gy) typically administered to these patients. Consistent with this finding, 21% of dogs undergoing high-dose intraoperative RT (IORT) (>25 Gy) followed by external-beam RT developed OSA following treatment, suggesting that the high dose per fraction and/or total dose may predispose to tumorigenesis (Powers et al. 1989).

Physical risk factors implicated in the initiation of OSA in dogs include heavy weight bearing to “sensitive” metaphyseal sites and malignant transformation around metallic implants (1/10,000 fracture repairs) or previous fractures (Gellasch et al. 2002; Stevenson et al. 1982). Rapidly proliferating cells may be more susceptible to oncogenic agents and mitotic errors leading to transformation. Other bone conditions associated with an increased risk of OSA development in dogs and humans involve bone changes caused by chronic osteomyelitis or bone infarcts. In humans, Paget disease accounts for >20% of OSA in patients older than 40 years of age (Ottaviani and Jaffe 2009). Paget’s disease is a premalignant condition characterized by excessive bone production, and approximately 1% of patients with the disease develop OSA. Paget OSAs are biologically aggressive, high-grade sarcomas, with up to 25% of patients presenting with metastasis at the time of initial presentation. Although Paget’s disease is not recognized in dogs, OSA has been seen concurrently in dogs with bone infarcts (Dubielzig et al. 1981; Marcellin-Little et al. 1999; Riser et al. 1972). It is unclear whether there is any causal relationship linking bone infarction to OSA in dogs; therefore, the contribution of Paget’s

disease to the pathogenesis of human OSA may be an important clinical distinction from canine OSA.

Genetic Factors

Consistent with the experience in human OSA, canine OSA is characterized by markedly abnormal karyotypes that may contain complex structural changes (translocations and/or rearrangements) and DNA copy number changes (Maeda et al. 2012; Mayr et al. 1991; Selvarajah et al. 2008; Thomas et al. 2009). Marked aneuploidy and karyotypic complexity has made it challenging to determine whether recurrent chromosomal aberrations characterize OSA and suggest that early oncogenic events in this cancer may result from alterations in DNA repair and sensing mechanisms, disturbances in chromosomal segregation mechanisms, or as a consequence of sentinel events such as chromothripsis.

Perhaps the most thoroughly described genetic alterations in human and canine OSA are mutation of the *p53* tumor suppressor gene and aberrant *RB1* gene signaling. Approximately 60% of canine OSA cell lines overexpress p53 protein, and this correlates with the presence of missense point mutations within the DNA-binding domain (Levine and Fleischli 2000). Corroborating these in vitro findings, mutations and overexpression of p53 protein in primary canine OSA have been reported in 41% and 67% of primary canine OSA tumors, respectively (Kirpensteijn et al. 2008; Loukopoulos et al. 2003). In human OSA, *p53* mutations occur in approximately 20% of cases (Wunder et al. 2005); however, these mutations frequently include genomic deletions, whereas canine *p53* mutations are largely restricted to point mutations (74%), with a smaller percentage of mutations (26%) being deletions (Kirpensteijn et al. 2008). Loss of heterozygosity of the *p53* gene also has been reported in 42% of human OSA tumors. In contrast, microarray-based comparative genomic hybridization (aCGH) studies of canine OSA found loss of heterozygosity of the *p53* gene in only 18% of canine tumors. Interestingly, genomic loss of *CDKN2A* occurred in additional cases that showed intact *p53* copy number, suggesting that global disruption of the p53 pathway may also play a role in canine OSA (Thomas et al. 2009).

The frequency of *RB1* alterations in sporadic human OSA is approximately 30% to 75% (Ottaviani and Jaffe, 2009). The importance of *RB1* gene dysregulation in canine OSA has been questioned based on early studies demonstrating lack of gross *RB1* gene alterations and normal protein expression in canine tumor samples (Mendoza et al. 1998). More recent studies have identified DNA copy number loss involving the *RB1* gene in 29% of canine OSA tumors, resulting in a correlative reduction or absence of RB1 protein expression in 62% samples tested (Thomas et al. 2009). These findings suggest that aberrations in the *RB1* gene in canine OSA parallel those found in humans, implicating *RB1* dysregulation in the formation and/or progression of OSA in both species.

In addition to *p53* and *RB1* gene abnormalities, genomic loss of the phosphatase and tensin homolog (*PTEN*) tumor

suppressor gene has been documented in human and canine OSA. In humans, homozygous gene deletion and consequent decrease in *PTEN* expression is a frequent event in OSA (Freeman et al. 2008). Consistent with this finding, canine OSA cell lines have demonstrated *PTEN* deletions and reduced protein expression (Levine et al. 2002), and more recent data from aCGH studies indicate that copy number loss involving the *PTEN* gene locus is a common event in canine OSA, present in 30% to 42% of tumors analyzed (Angstadt et al. 2011; Thomas et al. 2009).

Perhaps the most compelling data regarding the etiopathogenesis of OSA relate to humans with germline genetic alterations that lead to disease predisposition. Individuals with hereditary retinoblastoma associated with a germline mutation in the *RBI* gene commonly develop secondary malignancies, 40% of which are OSA (Gorlick 2009). Similarly, Li-Fraumeni syndrome is an autosomal dominant disorder caused by a mutation in the *p53* gene, and it has been found in up to 3% of children with OSA (McIntyre et al. 1994).

Although counterparts to these human genetic disorders have not been identified in dogs, specific breeds, including Scottish deerhounds, Rottweilers, greyhounds, Great Danes, Saint Bernards, and Irish wolfhounds, are at disproportionate risk for the development of OSA, and there is a growing body of evidence that supports breed-associated inheritance of risk factors associated with OSA. Selective breeding practices have narrowed the genetic diversity in domestic dog breeds, providing a unique opportunity to more clearly elucidate the hereditary basis for the formation of OSA in this species. Breed-specific gene expression signatures and specific recurrent cytogenetic aberrations, such as loss of *WT1*, occur exclusively or more frequently in Rottweilers than in other dogs (Scott et al. 2011; Thomas et al. 2009). Furthermore, a novel germline mutation in the receptor tyrosine kinase (RTK) *MET*, a receptor known to be dysregulated in both dogs and human OSA (MacEwen et al. 2003; Patane et al. 2006), has been identified primarily in the Rottweiler breed (Liao et al. 2006). Whole genome mapping approaches have identified a novel locus for OSA formation in the Scottish deerhound to CFA34 (a region syntenic to human chromosome 3q26), providing novel insight into the genetic basis of OSA in this breed. Recently, genome-wide association analysis in three breeds (Greyhound, Rottweiler, and Irish wolfhound) identified 33 inherited risk loci explaining 55% to 85% of phenotype variance in each breed. The greyhound locus exhibiting the strongest association (located 150 kb upstream of the genes *CDKN2A/B*) was also the most rearranged locus in canine OSA tumors (Karlsson et al. 2013). Importantly, despite the genetic complexity of OSA, mapping of multiple dog breeds revealed a polygenic spectrum of germline risk factors and identified candidate pathways as drivers of OSA.

Molecular Biology of OSA

The genetic instability and karyotypic complexity that is a hallmark of OSA has hindered identification of genetic

aberrations leading to OSA that drive tumor development and growth. In addition, the rarity of the disease in humans complicates the ability to discover relevant candidate genes. Until recently, the paucity of resources for canine genomic studies limited the scope of work that could be undertaken to profile genomic instability in the canine disease. The development and release of an annotated canine genome assembly has facilitated the development of genome integrated molecular reagents (Thomas et al. 2007) and commercially available high-throughput methodologies specific for dogs. Significantly, the development of molecular tools in both dogs and humans has enhanced our ability to use comparative genomics to characterize shared abnormalities in this complex disease. Cross-species comparative analyses found a strong similarity in the global gene expression patterns in canine and pediatric OSA (Paoloni et al. 2009; Scott et al. 2011). Cluster analysis of orthologous gene signatures did not segregate canine and human OSA on the basis of species, suggesting that cancers from each species are indistinguishable by gene expression analyses. Similarly, studies utilizing aCGH identified recurrent high-frequency DNA copy number aberrations in spontaneously arising canine OSA that are orthologous to regions of recurrent genome imbalance identified in human OSA (Angstadt et al. 2011; Angstadt et al. 2012). The use of dogs in cross-species genomic evaluation of complex cancers such as OSA provides an opportunity to identify regions of shared genomic instability among a background of unshared “noise.” Furthermore, the organization of dogs into defined breeds provides for a more homogenous genetic background, and this narrow genetic diversity enhances the ability to identify novel genetic alterations or molecular subtypes in OSA. For example, Paoloni et al. showed that expression of candidate “dog-like” genes *IL-8* and *SLC1A3*, which are overexpressed in canine OSA but have variable and/or low expression in human OSA, was associated with an aggressive clinical course and poor outcome in human OSA patients (Paoloni et al. 2009).

A fairly extensive list of genes altered in human and canine OSA has now emerged, precluding a comprehensive discussion of their altered functions in this article. Table 2 summarizes the molecular and genetic factors implicated in canine OSA and highlights those found to be similarly dysregulated in human OSA. Several genes and signaling pathways that are relevant targets for therapeutic intervention in canine and human OSA will be discussed below.

A major effort of comparative oncology has been to identify shared targets in human and canine OSA. Substantial progress has been made through experimental and preclinical investigations to identify dysregulated intracellular signaling pathways likely to contribute to the pathogenesis of this disease. For example, both canine and human OSA cell lines express the RTK *MET* and exhibit scattering, invasion, and enhanced migration in response to stimulation with ligand (hepatocyte growth factor) (Liao et al. 2005; MacEwen et al. 2003). Aberrant expression of *MET* has been demonstrated in canine OSA samples (Fieten et al. 2009; Raffaella et al. 2009), and in vitro work with the novel small molecule

Table 2 Molecular and Genetic Factors Associated with Canine Osteosarcoma

Factor*	Functions in Canine OSA
<i>p53</i>	<i>p53</i> mutated and overexpressed in OSA cell lines and primary tumors (Kirpensteijn et al. 2008; Levine and Fleischli, 2000) Loss of heterozygosity of <i>p53</i> in OSA tumors (Thomas et al. 2009)
<i>RB</i>	<i>RB1</i> copy number loss and reduced or absent <i>RB1</i> protein expression in OSA tumors (Thomas et al. 2009)
<i>PTEN</i>	<i>PTEN</i> deleted and down-regulated in OSA cell lines (Levine et al. 2002) <i>PTEN</i> copy number loss in OSA tumors (Angstadt et al. 2011; Thomas et al. 2009)
<i>MYC</i>	<i>MYC</i> copy number gain in OSA tumors (Thomas et al. 2009) <i>MYC</i> amplification detected in high percentage of Rottweiler OSA tumors
<i>CDKN2A/B</i>	Inherited risk gene loci (150 kb upstream of the genes <i>CDKN2A/B</i>) identified in OSA tumors from high-risk breeds (Karlsson et al. 2013)
<i>ErbB-2/HER-2</i>	<i>HER2</i> mRNA overexpressed in OSA cell lines and tumors (Flint et al. 2004)
<i>IGF-1/IGF-1R</i>	<i>IGF-1/IGF-1R</i> expressed in OSA cell lines; enhanced anchorage independent growth and invasion in response to <i>IGF-1</i> (MacEwen et al. 2004)
<i>RON/Met/EGFR</i>	<i>MET</i> expressed in OSA cell lines; enhanced invasion/migration in response to stimulation with ligand (<i>HGF</i>) (Liao et al. 2005) <i>MET</i> expressed in OSA tumors (Fieten et al. 2009; Raffaella et al. 2009) <i>EGFR</i> and <i>Ron</i> expressed in OSA cell lines and tumors; co-association of <i>Met</i> with <i>EGFR</i> and <i>Ron</i> in OSA cell lines (McCleese et al. 2013)
<i>STAT3</i>	Constitutive activation of <i>STAT3</i> in OSA tumors and cell lines; enhanced survival and proliferation in OSA cell lines (Fossey et al. 2010) Oncostatin M expressed in OSA tumors and promotes <i>STAT3</i> activation, <i>VEGF</i> production and invasion in OSA cell lines (Fossey et al. 2011)
<i>mTOR</i>	<i>mTOR</i> activation in canine OSA cell lines; inhibition of <i>mTOR</i> pathway decreases cell survival (Gordon et al. 2008)
<i>ezrin</i>	High <i>ezrin</i> expression in OSA tumors associated with early metastasis (Khanna et al. 2002) Activation of <i>ezrin</i> through <i>PKC</i> enhances cell migration (Hong et al. 2011)
<i>PDGFs/ PDGFRs</i>	<i>PDGF-A/B</i> and <i>PDGFRα/β</i> expressed in OSA tumors; <i>PDGFR α/β</i> and <i>PDGF-A</i> overexpressed in OSA cell lines (Maniscalco et al. 2013)
<i>MMPs</i>	OSA cell lines express high levels of <i>MMP-2/-9</i> (Loukopoulos et al. 2004)
<i>miR-134</i> <i>miR-544</i>	Decreased expression of <i>miR-134</i> and <i>miR-544</i> (orthologous to the human 14q32 miRNA cluster) in OSA tumors associated with shorter survival (Sarver et al. 2013)

*Bold indicates molecular and genetic factors similarly altered in human OSA

MET inhibitor PF2362376 in canine OSA cells lines supports the notion that *MET* is a relevant target for therapeutic intervention in OSA (Liao et al. 2007). In human cancers, coexpression and heterodimerization of the RTKs *MET*, epidermal growth factor receptor (*EGFR*), and *Ron* alters signal transduction and promotes resistance to targeted therapeutics. Similarly, canine OSA cell lines and primary tumors also demonstrate expression and phosphorylation of *EGFR* and *Ron*, and *MET* is coassociated with *EGFR* and *Ron* in canine OSA cells lines (McCleese et al. 2013). Furthermore, combination treatment of OSA cell lines with gefitinib and crizotinib inhibited cell proliferation in an additive manner. Together, these findings suggest receptor cross-talk in canine OSA, further supporting the targeting of *MET*, *EGFR*, and *Ron* interactions as a therapeutic strategy.

Constitutive activation of signal transducer and activator of transcription 3 (*STAT3*) is present in a subset of canine OSA

tumors and human and canine OSA cell lines, but not normal canine osteoblasts. More recent studies have found human OSA patients whose tumors express high levels of phospho-*STAT3* have a worse prognosis, providing further support for the idea that *STAT3* activation may be an important regulator of aggressive biologic behavior in OSA (Ryu et al. 2011; Wang et al. 2011). In addition, expression profiling of pediatric OSA revealed that tumors with a poorer prognosis were associated with greater expression of genes enhancing cell migration and remodeling, many of which are transcriptionally regulated by *STAT3* (Mintz et al. 2005). In both canine and human OSA cell lines, downregulation of *STAT3* activity through inhibition of upstream *Src* family kinases, inhibition of *STAT3* DNA binding and transcriptional activities, or modulation of *STAT3* expression resulted in decreased cell proliferation and viability, induction of apoptosis, and downregulation of known transcriptional targets

of STAT3 (Fossey et al. 2009). Therapies targeting STAT3 activation in OSA have been investigated in canine OSA cell lines, including the novel curcumin analog FLLL32, which decreased STAT3 DNA binding activity and expression and induced apoptosis in OSA tumor cell lines (Fossey et al. 2011). Studies evaluating the biologic activity of the novel allosteric small molecule STAT3 inhibitor LLL12 in canine OSA cell lines found that treatment with this drug inhibited proliferation, induced apoptosis, reduced STAT3 phosphorylation, and decreased the expression of several transcriptional targets of STAT3 in these cells (Couto et al. 2012). In addition, LLL12 exhibited synergistic antiproliferative activity with the cytotoxic chemotherapeutic doxorubicin in the OSA cell lines. Consistent with these findings in canine OSA cell lines, STAT3 inhibition via a novel small molecule STAT3 inhibitor (STA-21) or a dominant negative Stat3 Y705F resulted in inhibition of proliferation and apoptosis of human sarcoma cell lines expressing high levels of phospho-STAT3 (Chen et al. 2007). In addition, inhibition of STAT3 by LLL12 and FLLL32 in human OSA cells and murine xenograft models demonstrate that constitutive STAT3 signaling is required for OSA survival and migration in vitro and tumor growth in vivo (Onimoe et al. 2012). Together, these data support STAT3 as a relevant target for therapeutic intervention in OSA and support the clinical development of these drugs for the treatment of OSA.

The membrane-cytoskeleton linker ezrin mediates early metastatic survival, and its high expression in canine OSA tumors is associated with early development of metastases (Khanna et al. 2004). Consistent with data in dogs, there is a significant association between high ezrin expression and poor outcome in pediatric OSA patients. In vitro data in canine OSA cell lines found a relationship between PKC and ezrin-radixin-moesin in these cells and showed that ezrin phosphorylation and tumor cell migration were inhibited using a PKC inhibitor (Hong et al. 2011). Furthermore, p-ezrin-radixin-moesin overexpression occurred early in the development of pulmonary OSA micrometastases in an orthotopic xenograft mouse model of canine OSA but decreased at later time points, supporting the idea that ezrin contributes to the survival of cancer cells after their arrival at secondary metastatic locations (Jaroensong et al. 2012). The development of metastasis is the most significant cause of death in humans and dogs with OSA; therefore, the discovery of novel therapeutics to prevent tumor metastasis is an active area of research. Small molecule inhibitors of ezrin are currently under development, and early in vitro and in vivo data demonstrate biologic activity and inhibition of the invasive phenotype in OSA cells (Bulut et al. 2012). One important advantage of dogs as models of human cancer is the ability to test novel therapeutic agents in the setting of minimal residual disease. Given the association between ezrin activation and the metastatic phenotype in human and canine OSA, inclusion of dogs with spontaneously occurring disease in the development and testing of therapeutics that target ezrin biology will likely provide important new information with direct relevance to future testing in people.

Pathophysiology and Natural Behavior of OSA

Histopathological Subtypes of OSA

OSA arises from a mesenchymal stem cell that has or can acquire the capacity to produce osteoid. Historically, it has been believed to develop from an osteoblast, but given that these tumors are capable of differentiating toward fibrous tissue, cartilage, or bone and can have chondroblastic, fibroblastic, and osteoblastic components, the cell of origin may have a more pluripotent potential and thus derive from a more primitive precursor (Gorlick 2009; Wilson et al. 2008).

In both human and canine OSA, tumors are classified based on location, cell type (representing >50% of the malignancy), and tumor grade. The majority of canine tumors are located in long bones of the appendicular skeleton and represent high-grade, osteoblastic osteoid-producing malignancies, but they may be histologically subclassified into chondroblastic, fibroblastic, or telangectatic subtypes. Similarly, the vast majority of human OSAs are high-grade, osteoblastic OSA arising in similar locations to those in dogs. In both species, histologic subtype does not appear to influence biologic behavior; however, high histologic grade, based on microscopic features (cellular pleiomorphism, mitotic index, tumor matrix, and percent necrosis), is associated with poor clinical outcome (Kirpensteijn et al. 2002; Klein and Seigal 2006).

Clinical Behavior of OSA

The parallels between canine and human OSA are significant in their clinical presentation, biologic behavior, histology, and conventional and investigational treatments. The primary differences between the diseases in the two species are the high prevalence of OSA in dogs, the relative age of onset (canine OSA is a disease of adulthood, whereas humans are commonly affected as adolescents), and the poorer outcomes in dogs. Most human and canine patients with OSA present with a history of lameness and swelling at the primary site. Due to the locally aggressive nature of OSA, soft tissue swelling and pathologic fracture of the affected bone can occur. Radiographic findings in canine and human OSA are virtually indistinguishable, with tumors demonstrating a classically described “sunburst” pattern of mixed bone lysis and new bone (tumor or reactive bone) formation and periosteal elevation related to a soft tissue mass (referred to as “Codman’s triangle”) (Gorlick and Khanna 2010; Ehrhart et al. 2013).

A defining feature of OSA is the high rate of metastasis that results from the primary bone tumor disseminating via hematogenous spread to distant secondary sites. The majority of deaths that occur due to disease in both canine and human OSA patients are due to the development of metastasis, primarily to the lungs, and, less commonly, to other bones or soft tissues. Although <15% of canine and human patients have radiologic evidence of metastases at diagnosis, 85% to

90% of patients develop gross metastases despite effective control of the primary bone tumor, indicating that subclinical micrometastases arise early in the course of the disease (Ehrhart et al. 2013; Gorlick and Khanna 2010). The adoption of chemotherapy protocols and aggressive surgical techniques has improved survival; however, the overall 5-year survival rate for OSA in humans is approximately 60% to 70% in the nonmetastatic disease setting and 10% to 30% if metastases are found at initial diagnosis (Allison et al. 2012; Bielack et al. 2002; Harris et al. 1998; Meyers et al. 2005). In contrast, treatment of dogs with OSA remains minimally effective and results in long-term survival rates of only 10% to 15% (Withrow et al. 1991), suggesting that the canine disease exhibits a more aggressive biologic behavior. In human OSA, the importance of the dose intensity (DI) of neoadjuvant chemotherapy protocols is controversial (Bacci et al. 2001; Eselgrim et al. 2006; Lewis et al. 2007), but several studies have identified a strong association between increased DI of methotrexate and doxorubicin and survival in human OSA (Delepine et al. 1996; Kawai et al. 1996). Although a reduced DI may account for some of the observed differences in outcome in dogs with OSA treated with chemotherapy when compared with humans, a recent study investigating the association of DI with disease-free interval (DFI) and overall survival time in dogs with appendicular OSA treated with carboplatin or doxorubicin found no association between high summation DI and development of metastasis or overall survival (Selmic et al. 2014). These data suggest that other factors may contribute to the apparent aggressive behavior of OSA in dogs.

Prognostic Factors

Several well-documented prognostic indicators for canine and human OSA are strikingly similar. Interestingly, proximal humeral location is a significant negative prognostic factor in both canine and human appendicular OSA (Boerman et al. 2012; Cho et al. 2010; Kuntz et al. 1998; McMahan et al. 2011; Schmidt et al. 2013; Sottnik et al. 2010). Other prognostic factors associated with survival in dogs and humans with OSA include tumor location, presence of metastasis, the use of adjuvant and/or neoadjuvant chemotherapy, and postoperative infection at limb-sparing surgical sites.

In humans, the age of the patient, tumor size, local recurrence, and the degree of necrosis present in the resected tumor specimen after neoadjuvant therapy also have been correlated with overall survival. The poorest survival rates are reported among older individuals (>65 years of age); however, this may represent a second malignancy, likely related to Paget's disease (Ottaviani and Jaffe, 2009). Young dogs with OSA previously were reported to have shorter survival times (Spodnick et al. 1992); however, in contrast, a recent meta-analysis found that increasing age was associated with shorter survival time and DFI, but this was not statistically significant (Boerman et al. 2012). Similarly, one study in dogs evaluating quantitative bone scintigraphy to assess tumor size and

clinical outcome found that larger tumor area was associated with earlier metastasis (Forrest et al. 1992); however, nuclear scintigraphy is not routinely used, and more recent studies have demonstrated that this imaging technique significantly overestimates tumor length when compared with macroslide specimen measurements (Leibman et al. 2001). The degree of necrosis of resected tumor specimens in human OSA assists in determining response to neoadjuvant chemotherapy and is a significant prognostic factor in patients without evidence of metastatic disease. Patients are divided into good or poor responders based on their histologic response, and this correlates with subsequent disease-free survival following tumor removal and postoperative chemotherapy (Provisor et al. 1997). In contrast, neoadjuvant chemotherapy regimens are uncommonly employed in the treatment of canine OSA, and equivalent histologic grading schemes do not currently exist in veterinary medicine to predict outcome and guide postoperative chemotherapy.

Specific tumor-associated genetic determinants associated with clinical outcome and prognoses have been described recently in canine OSA. Gene expression profiling of primary OSA tissues identified prognostic gene profiles associated with DFI (deemed "good" or "poor" responders) and identified biologic pathways within the poor responder group involved in proliferation, drug resistance, and metastasis (O'Donoghue et al. 2010; Selvarajah et al. 2009). More recently, in an effort to uncover conserved gene expression patterns in canine and human OSA, genome-wide gene expression profiling was performed using early passage immortalized canine OSA cell lines derived from primary tumors that arose in high-risk breeds. When the molecular gene expression signatures derived from early passage cell lines were applied to primary canine OSA samples, samples were segregated into distinct molecular subgroups that predicted outcome. Most significantly, when the same genetic signatures identified in canine OSA were applied to available human OSA gene datasets, this allowed for prognostic molecular classification of the human tumors (Scott et al. 2011). These data indicate that cross-species genomic comparisons can aid in identifying discrete and reproducible molecular subtypes in OSA and that gene signatures uncovered by these studies may have clinical utility in predicting biologic behavior.

Current Treatment Options for Canine OSA

Surgical Amputation and Limb-Sparing Procedures

Therapy for OSA directed at the primary tumor involves surgical options such as amputation, limb-sparing procedures, or rotationplasty, the latter of which is performed exclusively in humans. Pre-, intra-, and postoperative interventions for dogs and humans are similar in terms of techniques, devices, allografts, clinical healing, and complications. These similarities, in combination with the parallels in current and developing

imaging techniques, provide additional support for the use of pet dogs with OSA in the assessment of preoperative and operative techniques to optimize limb-sparing surgical procedures commonly used in the management of pediatric patients. Early studies in large-breed dogs with OSA undergoing various limb-sparing surgical approaches provided meaningful data on limb-sparing techniques, bone allograft antigenicity, implant loosening and fracture, and durable allograft healing (LaRue et al. 1989; Stevenson et al. 1996, Straw et al. 1992). Importantly, the similarities between humans and dogs with respect to their size, tumor biology, and anatomy of the surgical site have been essential to engineering devices for limb sparing or prosthesis and optimizing surgical interventions that are difficult to recreate in other animal model systems (Paoloni and Khanna 2008; Withrow and Wilkins 2010).

Several different limb-salvage techniques have been described in dogs and have been utilized in human patients, including bone allografts (Liptak et al. 2006), ipsilateral vascularized ulnar transposition autografts (Seguin et al. 2003), pasteurized tumoral autografts (Morello et al. 2003), bone transport osteogenesis (Ehrhart 2005; Tommasini et al. 2000), and intraoperative radiation techniques (Boston et al. 2007; Liptak et al. 2004). Preoperative downstaging of the primary tumor with chemotherapy is not a common practice in dogs compared with humans; however, early in the development of limb-sparing procedures, several forms of preoperative treatment delivered by various methods were evaluated in dogs. These therapies included primary or neoadjuvant intra-arterial (IA) cisplatin, intravenous (IV) cisplatin, RT to the tumor, or a combination of RT with IV or IA cisplatin (Withrow et al. 1993). These studies demonstrated significant decreases in the degree of vascularization and a high degree of tumor necrosis in resected specimens in dogs receiving preoperative IA cisplatin, especially when combined with RT. Importantly, these data showed that the IA delivery system for cisplatin was technically feasible, safe, and effective in dogs and subsequently refined preoperative dosing strategies in humans. Studies of canine OSA also established a dose response for fractionated external beam radiation and indicated that the combination with cisplatin was additive, if not synergistic with radiation on local tumor cell kill (Withrow et al. 1993). Additionally, it was established that the treatment of dogs with RT alone given in large doses per fraction prior to limb sparing was unsatisfactory for preservation of life or limb (Thrall et al. 1990).

An unanticipated finding during early limb-sparing technique development was that dogs with allograft infections, which is a common and major complication related to limb-sparing surgery, experience a significant prolongation of overall survival time compared with dogs that do not develop infected allografts (Lascelles et al. 2005). This finding is reported in humans with deep infections following limb salvage surgery for OSA (Jeys et al. 2007) and appears to be independent of bacterial strain, severity (most are low grade), or duration (most are chronic) of infection. Proposed mechanisms include nonspecific immunologic stimulation,

antiangiogenic aspects of certain antibiotics, and host-versus-graft immune response to the allograft; however, the exact mechanisms remain to be elucidated.

The complex interaction of the immune system with tumor cells and the identification of populations of cells that have the ability to suppress antitumor immune responses have been challenging to dissect in animal models; however, regulatory T cells (Tregs) and myeloid-derived suppressor cells have been characterized in healthy and cancer-bearing dogs (Biller et al. 2007; Risetto et al. 2010; Sherger et al. 2012). Studies evaluating the clinical significance of Tregs in dogs with OSA found that the ratio of CD8⁺ T cells to Tregs in the blood is associated with overall survival time (Biller et al. 2010). Additional work found that shorter DFI was associated with relative lymphocytosis and relative monocytosis on initial bloodwork (Sottnick et al. 2010). These studies provide additional support for the notion that systemic antitumor activity plays an important role in the pathogenesis of OSA in dogs. Naturally occurring cancers in dogs spontaneously develop in the context of an intact immune system where tumor, host, and tumor microenvironment are syngeneic. To this end, the biologic similarities between canine and human OSA provide significant rationale for the study of novel immunomodulatory agents in dogs with OSA.

Systemic Adjuvant Therapy and Investigational Therapies for Metastatic Disease

In both humans and dogs, the most effective management of OSA involves the incorporation of multimodality therapy to address both the primary tumor and metastatic disease. Systemic chemotherapy remains the backbone for the management of metastasis; however, it is unlikely that new cytotoxic agents or dose intensification with existing agents will dramatically improve current clinical outcomes. In dogs, platinum-based (cisplatin, carboplatin) chemotherapy either alone or in combination with doxorubicin has been demonstrated to improve survival after amputation. Currently, the standard treatment for this disease in dogs involves either single agent or multi-agent chemotherapy, but despite this aggressive approach, >50% of dogs do not live beyond 1 year postamputation and 90% die of disease by 2 years (Ehrhart et al. 2013). The combination of high-dose methotrexate, doxorubicin, and cisplatin constitutes the standard therapeutic approach for OSA in people in both the United States and Europe (Rainusso et al. 2013). A variety of clinical trials has been completed in both dogs and humans in an attempt to improve outcomes, yet to date, none have proven successful.

Clinical trials evaluating the anticancer immune effects associated with the administration of liposome-encapsulated muramyl tripeptide-phosphatidylethanolamine (L-MTP-PE) were conducted in dogs with OSA. L-MTP-PE is a lipophilic derivative of muramyl dipeptide, a synthetic analog of a mycobacterium cell wall component. Initial clinical evaluation of L-MTP-PE administered to dogs in the setting of minimal

residual disease (immediately following amputation) demonstrated single-agent anticancer activity (MacEwen et al. 1989). Subsequent randomized, placebo-controlled clinical trials of L-MTP-PE conducted in conjunction with standard-of-care chemotherapy in dogs (Kurzman et al. 1995) were part of the scientific rationale for phase III evaluation of L-MTP-PE in pediatric OSA. Findings of a Children's Oncology Group clinical trial were similar to those reported in the initial canine studies and demonstrated that the addition of L-MTP-PE to standard chemotherapy in pediatric OSA significantly improved overall survival at 6 years (Meyers et al. 2008). Based on these findings, L-MTP-PE (mifamurtide, Mepact) has recently been approved for the treatment of metastatic OSA by the European Medical Association, highlighting the utility of pet dogs with spontaneously occurring cancers for the investigation of novel therapeutic agents in the setting of minimal residual disease.

Several molecular therapies with the capacity to delay or inhibit the development of pulmonary metastasis have been evaluated in dogs. In osteoblasts, IGF-1 induces cell mitogenesis and protection from apoptosis as well as promotes angiogenesis. Human and canine OSA cell lines express both IGF-1 and IGF-1 receptor, proliferate in response to IGF-1, and demonstrate an antiapoptotic phenotype in vitro after IGF-1 exposure (Bostedt et al. 2001; MacEwen et al. 2004). Inhibition of IGF-1/IGF1-R1 signaling in human OSA cell lines with a novel small molecule inhibitor (OSI-906) or lentivirus-mediated RNAi knockdown of IGF1-R1 decreased cell proliferation and reduced invasion, and enhanced radiosensitivity, respectively (Kuijjer et al. 2013; Wang et al. 2009). Furthermore, the expression of IGF1-R1 was found to be closely associated with surgical stage, distant metastasis, and poor survival in human patients with OSA, suggesting that therapeutic targeting of the IGF-1 pathway may be an effective treatment for OSA metastases (Wang et al. 2012). A randomized clinical trial in dogs with OSA was conducted where dogs undergoing standard amputation were administered postoperative carboplatin chemotherapy with either a long-acting analog of somatostatin (OncoLAR), which attenuates the protumorigenic effects of IGF-1 through inhibition of growth hormone and/or growth hormone-releasing hormone, or placebo-control (Khanna et al. 2002). Circulating IGF-1 concentrations were measured throughout therapy, and administration of OncoLAR resulted in significant suppression in IGF-1 concentrations compared with baseline values; however, this finding did not translate to improved DFI or overall survival compared with dogs receiving placebo.

Alternative approaches for the treatment of gross metastatic disease have been explored in dogs with OSA using RTK inhibitors or the localized delivery of prostimulatory cytokines to augment antitumor immunity. Toceranib phosphate (Palladia), a multi-targeted RTK inhibitor, has demonstrated preliminary anticancer activity in metastatic pulmonary OSA in dogs (London et al. 2012). Targets of toceranib include several members of the split-kinase family such as vascular endothelial growth factor receptor, platelet derived growth factor receptor, and KIT. Although the exact mechanism

through which toceranib exerts its activity on metastatic pulmonary OSA is unknown, several proposed mechanisms include antiangiogenic activity through modulation of vascular endothelial growth factor receptor and platelet derived growth factor receptor or through enhanced antitumor immune response by decreasing Treg numbers (Mitchell et al. 2012). Other investigational therapies have evaluated the antitumor activity of liposomal IL-2 delivered directly to the pulmonary parenchyma of dogs with gross metastatic OSA in the form of inhaled nebulization therapy (Khanna et al. 1997). This study demonstrated evidence of local immunomodulatory effects of IL-2, and several durable clinical responses were documented in dogs with macroscopic pulmonary OSA. This proof-of-concept study in dogs established a safety and efficacy profile for inhaled liposomal IL-2 therapy and launched subsequent investigations evaluating alternative drug delivery strategies, such as IV gene therapy using liposome-DNA complexes encoding the canine *IL-2* gene (Dow et al. 2005).

RT and Palliative Treatments

The utility of RT in the treatment of appendicular OSA in dogs and humans continues to evolve. In dogs, the most common role of RT is for palliation of bone pain; however, several veterinary studies have evaluated the use of extracorporeal IORT techniques for limb sparing, stereotactic radiosurgery as a nonsurgical limb-sparing alternative, curative-intent RT protocols for local tumor control, and the use of bone-seeking radioisotopes for treatment of primary OSA and metastatic bone lesions.

The IORT technique for limb sparing has been utilized in a small number of canine OSA patients (Liptak et al. 2004) as well as in human patients with extremity bone tumors (Oya et al. 2001). The IORT technique has an advantage over surgical limb salvage procedures in preserving limb function in anatomic sites that are not amenable to reliable surgical limb salvage (e.g., proximal humerus); however, the high complication rate associated with orthopedic implant failure, pathologic fracture, and infection in the irradiated bone has precluded the use of this nonsurgical alternative to surgical limb sparing (Kuntz et al. 1998). Curative-intent RT protocols for local tumor control typically involve relatively high total doses of radiation, causing considerable necrosis of the tumor in dogs and humans, either before limb salvage to downstage the primary tumor or as a primary therapy for unresectable tumors (Machak et al. 2003; Walter et al. 2005). The introduction of stereotactic RT (SRT) holds promise in providing local tumor control for OSA. SRT delivers high-dose RT to the tumor volume with relative sparing of the surrounding normal tissues by use of image guidance and a sharp drop off in dose intensity. Access to equipment has hindered progress in evaluating SRT treatment protocols in canine OSA; however, initial reports using SRT with or without systemic chemotherapy demonstrated long-term local disease control in several dogs and improved limb function in all dogs treated (Farese et al. 2004). Further evaluation of SRT techniques alone or in

combination with other bone-targeted therapeutics in canine OSA provides an opportunity to refine dosing strategies, identify short- and long-term complications associated with therapy, and determine the viability of SRT as a nonsurgical limb-sparing alternative for local tumor control in human and canine OSA.

RT is considered the most effective treatment modality for the management of osteolytic bone pain in human cancer patients and likewise has been investigated and extensively applied to alleviating bone cancer pain in dogs with OSA. Malignant osteolytic pain is a major source of morbidity in canine and human cancer patients and has a significant impact on quality of life. Strategies to effectively manage this pain in dogs with OSA include the systemic administration of conjugated radiopharmaceuticals targeted to areas of increased osteoblastic activity (Samarium-153-EDTMP) and aminobisphosphonates. Bone-targeted RT with Samarium-153-EDTMP has been used in human patients for the palliation of pain associated with various metastatic bone neoplasms (Anderson and Nuñez 2007). Studies evaluating the efficacy and clinical response of canine primary bone tumors treated with Samarium-153-EDTMP have demonstrated that high doses can be deposited preferentially within tumor tissue (Aas et al. 1999). Furthermore, one study found that 63% of dogs with appendicular OSA treated with Samarium-153-EDTMP showed improvement in the severity of their lameness 2 weeks after administration of their first treatment (Barnard et al. 2007). Although the rationale for evaluating aminobisphosphonate in the management of bone pain in dogs with OSA was based on the historical use of these agents for the treatment of malignant osteolysis in humans, early studies establishing the safety of single-agent IV pamidronate in dogs with OSA found that dogs treated with pamidronate achieved subjective pain alleviation, and this correlated with changes in urine N-telopeptide (NTx) concentrations and relative primary tumor bone density measurements (Fan et al. 2007). Subsequent prospective, double-blind, randomized, placebo-controlled clinical trials in dogs with appendicular OSA found that the addition of pamidronate to palliative RT appears to improve limb function in dogs when compared with palliative RT alone (Ryan et al. 2011). Canine studies have now established several subjective and quantitative end points to aid in the investigation of clinically effective agents for the management of malignant bone pain. These include imaging modalities such as dual-energy x-ray absorptiometry to assess relative primary tumor bone mineral density, force plate gait analysis and numerical lameness evaluation to assess limb function, commercially available assays to detect bone resorption markers in urine that have been validated in dogs (Fan et al. 2005; Lucas et al. 2008), and the establishment of the Canine Brief Pain Inventory, which is based on the Brief Pain Inventory used in people with bone cancer (Brown et al. 2009). The establishment of clinically relevant end points in dogs with malignant osteolytic pain highlights the opportunity to integrate pet dogs into clinical trials evaluating novel therapeutic agents for the management of malignant bone pain in humans.

Dog Model for Development of Novel and Translational Therapies for OSA

Studies in dogs are uniquely positioned to evaluate the efficacy and feasibility of novel drugs and drug delivery devices and can inform the go/no-go “decision gate” in clinical drug development. To this end, a comparative study in dogs was conducted to evaluate the safety, efficacy, and feasibility of aerosolized gemcitabine in the management of macroscopic pulmonary OSA metastasis (Rodriguez et al. 2010). This method of drug delivery was based on preclinical data from mouse OSA xenograft models that demonstrated the anticancer activity of aerosolized gemcitabine was mediated through upregulation of Fas receptor expression on the surface of metastatic tumor cells in the lungs (Koshkina and Kleinerman 2005). Data from canine studies demonstrated that aerosolized gemcitabine was well tolerated with no dose-limiting hematologic or biochemical toxicity and minimal histologic lung pathology following inhalation therapy in dogs with macroscopic lung metastases. Although clinically relevant reductions in tumor size were not achieved, identification of increases in the percent necrosis and Fas receptor expression in the metastatic lesions supported the proposed mechanism of antitumor activity associated with this therapy in dogs and contributed to the clinical development of inhalation approaches in humans (Rodriguez et al. 2010). Ongoing strategies targeting the Fas receptor/Fas ligand in OSA continue to be investigated in dogs, including intratumoral Fas ligand gene therapy delivery (Modiano et al. 2012).

Clinical trials in dogs with OSA also have aided in establishing relationships between a cancer target, its modulation with a small-molecule inhibitor, and clinical benefit. Correlative studies that would be challenging to conduct in humans, including multiple biopsy and collection time points, are feasible in pet dog studies. A prospective dose escalation study of rapamycin in dogs with OSA was performed to define optimal dosing schedules, biomarkers, and rationale for the use of rapamycin or potentially other mTOR inhibitors in OSA (Paoloni et al. 2010). Pre- and posttreatment biopsies and peripheral blood mononuclear cells (PBMCs) were collected, and 48-hour whole blood sampling was performed to establish a pharmacokinetically relevant and pharmacodynamically active dose of rapamycin in dogs with OSA. Data from this phase I trial demonstrated that biologically effective concentrations of rapamycin were safely obtainable in dogs and provided evidence of target modulation in tumor tissues and PBMCs. This study highlights the advantage of integrating the comparative approach in the development path of new cancer drugs. Importantly, such studies help to establish critical pharmacokinetic and pharmacodynamic relationships, so that drugs with an unfavorable therapeutic index or inferior target modulation attributes may be identified and removed from development earlier in the process, thus identifying agents most likely to succeed in human clinical trials (Gordon et al. 2009; Gordon and Khanna 2010).

The evaluation of novel therapeutic approaches in dogs with spontaneous cancer can have significant impact on

Table 3 Studies Conducted in Dogs that have Informed the Management of Human OSA

	Canine Studies	Human Studies
Prognostic factors	<i>IL-8</i> and <i>SLC1A3</i> overexpression is associated with aggressive clinical behavior and poor outcome in canine OSA (Paoloni et al. 2009)	Serum IL-8, and TNF- α levels are associated with increased risk and progression of OSA (Xiao et al. 2013)
Targeted therapeutics	Phase I trial of rapamycin in dogs with OSA defined optimal dosing schedules, biomarkers and rationale for the use mTOR inhibitors in OSA (Paoloni et al. 2010)	Phase II study of mTOR inhibitor, Ridaforolimus demonstrated single-agent activity in advanced sarcomas (Chawla et al. 2012) Phase III trial of Ridaforolimus maintenance therapy in metastatic sarcomas delays tumor progression (Demetri et al. 2013)
Immunotherapy	Randomized, double-blind clinical trials of L-MTP-PE conducted in conjunction with standard-of-care chemotherapy in dogs with appendicular OSA demonstrated that L-MTP-PE has antimetastatic activity in dogs with OSA when given following amputation (Kurzman et al. 1995; MacEwen et al. 1989)	The addition of L-MTP-PE to standard multi-drug chemotherapy in human OSA significantly improved overall survival and disease-free interval (Meyers et al. 2005; Meyers et al. 2008)
Chemotherapy delivery	Preoperative intra-arterial (IA) delivery system for the administration of cisplatin to dogs with OSA was technically feasible, safe, and resulted in significant decreases in the degree of vascularization and a high degree of tumor necrosis (Withrow et al. 1993)	Protocols using a combination of IA cisplatin and IV doxorubicin have resulted in significant histologic response rate, improved disease-free and overall survival in pediatric and adult OSA (Cullen et al. 2005; Wilkins et al. 2005)
Limb-sparing techniques	Limb-sparing surgical approaches performed in large-breed dogs with OSA provided meaningful data on limb-sparing techniques, bone allograft antigenicity, implant loosening and fracture, and durable allograft healing (LaRue et al. 1989; Stevenson et al. 1996, Straw et al. 1992).	Several decades ago, amputation was considered the only option for local tumor control, but limb-salvage surgery has become an accepted treatment standard in the management of patients with OSA (Mangat et al. 2011; Rainusso et al. 2013)

informing the development and conduct of later stage studies in humans. Heat shock protein 90, a molecular chaperone that promotes the conformational maturation and stabilization of a wide variety of client proteins, is a promising target for therapeutic intervention in cancer. Ganetespib, a novel small molecule inhibitor of Heat shock protein 90, and its water soluble prodrug, STA-1474, demonstrated activity against canine OSA cell lines in vitro. Consistent with these findings, STA-1474 induced tumor regression, apoptosis, and down-regulation of key targets including MET and AKT in OSA xenografts (McCleese et al. 2009). Based on these findings, a Phase I study of STA-1474 was performed in dogs with spontaneous tumors (London et al. 2011). This clinical trial demonstrated biologic activity in various canine cancers (including dogs with metastatic OSA), established safety and toxicity profiles, and provided important information regarding expected gastrointestinal adverse events that were subsequently observed in human clinical trials with ganetespib. Pharmacodynamic end points provided evidence of target modulation in PBMCs and established this as a reliable biomarker of drug activity. Additionally, pharmacokinetic analysis in this study provided information on drug levels and exposure duration that subsequently established dosing

schemes that were unanticipated prior to the start of the clinical trial. These data laid the groundwork for the current clinical evaluation of ganetespib in humans.

Additional examples exist that highlight the use of dog models to inform preclinical drug development, including antiangiogenic agents (thrombospondin-1 peptide mimetics, ABT-526, and ABT-510; Rusk et al. 2006) and agents targeting pathways associated with invasion and metastasis (matrix metalloprotease inhibitor, BAY 12-9566; Moore et al. 2007). An advantage of the canine model of OSA is the ability to investigate the safety and efficacy of novel therapeutics in dogs as a prelude to future clinical work in humans. Importantly, the evaluation of novel therapeutics in dogs with OSA in the setting of minimal residual disease more accurately models the complex biology of OSA metastasis that is integral to outcomes in both human and canine patients. Table 3 provides a summary of key studies in which investigations performed in canine OSA have informed the management of human OSA.

Conclusions

The similarities between pediatric and canine OSA with regard to histology, biologic behavior, and molecular genetic

alterations indicate that canine OSA is a relevant, spontaneous, large animal model of the pediatric disease. Increasing awareness for the need for more useful animal models in human cancer drug development and the organization of a number of consortia and collective groups will aid in the effort to integrate dogs with OSA into comparative and translational cancer research. Comparative oncology approaches and cross-species genomic comparisons have the potential to identify shared and novel targets for therapeutic intervention in OSA. It is anticipated that the increasing availability of banked canine tumor specimens will allow for progress in identifying molecular signatures and valid tumor targets in canine OSA. The integration of pet dogs with OSA in pre-clinical studies within the development path of existing and novel cancer drugs has the potential to translate into more optimal design of human clinical trials and reduced late attrition or failure of cancer drugs in human patients. Ultimately, this comparative oncology effort will lend additional insight into the biology of OSA and lead to advancements in the care of both children and dogs affected by this disease.

References

- Aas M, Moe L, Gamlem H, Skretting A, Ottesen N, Bruland OS. 1999. Internal radionuclide therapy of primary osteosarcoma in dogs, using ¹⁵³Sm-ethylene-diamino-tetramethylene-phosphonate (EDTMP). *Clin Cancer Res* 5:3148s–3152s.
- Allison DC, Carney SC, Ahlmann ER, Hendifar A, Chawla S, Fedenko A, Angeles C, Menendez LR. 2012. A meta-analysis of osteosarcoma outcomes in the modern medical era. *Sarcoma* 2012:704872.
- Anderson P, Nuñez R. 2007. Samarium lexidronam (¹⁵³Sm-EDTMP): Skeletal radiation for osteoblastic bone metastases and osteosarcoma. *Expert Rev Anticancer Ther* 7:1517–27.
- Angstadt AY, Motsinger-Reif A, Thomas R, Kisseberth WC, Guillermo Couto C, Duval DL, Nielsen DM, Modiano JF, Breen M. 2011. Characterization of canine osteosarcoma by array comparative genomic hybridization and RT-qPCR: Signatures of genomic imbalance in canine osteosarcoma parallel the human counterpart. *Genes Chromosomes Cancer* 50:859–874.
- Angstadt AY, Thayanithy V, Subramanian S, Modiano JF, Breen M. 2012. A genome-wide approach to comparative oncology: High-resolution oligonucleotide aCGH of canine and human osteosarcoma pinpoints shared microaberrations. *Cancer Genet* 205:572–587.
- Bacci G, Ferrari S, Longhi A, Forni C, Giacomini S, Lari S, Versari M. 2001. Relationship between dose-intensity of treatment and outcome for patients with osteosarcoma of the extremity treated with neoadjuvant chemotherapy. *Oncol Rep* 8:883–888.
- Barnard SM, Zuber RM, Moore AS. 2007. Samarium Sm ¹⁵³ lexidronam for the palliative treatment of dogs with primary bone tumors: 35 cases (1999–2005). *J Am Vet Med Assoc* 230:1877–81.
- Bielack SS, Kempf-Bielack B, Delling G, Exner GU, Flege S, Helmke K, Kotz R, Salzer-Kuntschik M, Werner M, Winkelmann W, Zoubek A, Jurgens H, Winkler K. 2002. Prognostic factors in high-grade osteosarcoma of the extremities or trunk: An analysis of 1,702 patients treated on neoadjuvant cooperative osteosarcoma study group protocols. *J Clin Oncol* 20:776–790.
- Billar BJ, Elmslie RE, Burnett RC, Avery AC, Dow SW. 2007. Use of FoxP3 expression to identify regulatory T cells in healthy dogs and dogs with cancer. *Vet Immunol Immunopathol* 116:69–78.
- Billar BJ, Guth A, Burton JH, Dow SW. 2010. Decreased ratio of CD8+ T cells to regulatory T cells associated with decreased survival in dogs with osteosarcoma. *J Vet Intern Med* 24:1118–1123.
- Boerman I, Selvarajah GT, Nielen M, Kirpensteijn J. 2012. Prognostic factors in canine appendicular osteosarcoma: A meta-analysis. *BMC Vet Res* 8:56.
- Bostedt KT, Schmid C, Ghirlanda-Keller C, Olie R, Winterhalter KH, Zapf J. 2001. Insulin-like growth factor (IGF) I down-regulates type 1 IGF receptor (IGF 1R) and reduces the IGF I response in A549 non-small-cell lung cancer and Saos-2/B-10 osteoblastic osteosarcoma cells. *Exp Cell Res* 271:368–377.
- Boston SE, Duerr F, Bacon N, Larue S, Ehrhart EJ, Withrow SJ. 2007. Intra-operative radiation for limb sparing of the distal aspect of the radius without transcarpal plating in five dogs. *Vet Surg* 36:314–323.
- Brown DC, Boston R, Coyne JC, Farrar JT. 2009. A novel approach to the use of animals in studies of pain: Validation of the canine brief pain inventory in canine bone cancer. *Pain Med* 10:133–42.
- Bulut G, Hong SH, Chen K, Beauchamp EM, Rahim S, Kosturko GW, Glasgow E, Dakshanamurthy S, Lee HS, Daar I, Toretzky JA, Khanna C, Uren A. 2012. Small molecule inhibitors of ezrin inhibit the invasive phenotype of osteosarcoma cells. *Oncogene* 31:269–81.
- Chawla SP, Staddon AP, Baker LH, Schuetze SM, Tolcher AW, D'Amato GZ, Blay JY, Mita MM, Sankhala KK, Berk L, Rivera VM, Clackson T, Loewy JW, Haluska FG, Demetri GD. 2012. Phase II study of the mammalian target of rapamycin inhibitor ridaforolimus in patients with advanced bone and soft tissue sarcomas. *J Clin Oncol* 30:78–84.
- Chen CL, Loy A, Cen L, Chan C, Hsieh FC, Cheng G, Wu B, Qualman SJ, Kunisada K, Yamauchi-Takahara K, Lin J. 2007. Signal transducer and activator of transcription 3 is involved in cell growth and survival of human rhabdomyosarcoma and osteosarcoma cells. *BMC Cancer* 7:111.
- Cho WH, Song WS, Jeon DG, Kong CB, Kim MS, Lee JA, Yoo JY, Kim JD, Lee SY. 2010. Differential presentations, clinical courses, and survivals of osteosarcomas of the proximal humerus over other extremity locations. *Ann Surg Oncol* 17:702–708.
- Couto JI, Bear MD, Lin J, Pannel M, Kulp SK, Kisseberth WC, London CA. 2012. Biologic activity of the novel small molecule STAT3 inhibitor LLL12 against canine osteosarcoma cell lines. *BMC Vet Res* 8:244.
- Cullen JW, Jamroz BA, Stevens SL, Madsen W, Hinshaw I, Wilkins RM, Cullen P, Camozzi AB, Fink K, Peck SD, Kelly CM. 2005. The value of serial arteriography in osteosarcoma: Delivery of chemotherapy, determination of therapy duration, and prediction of necrosis. *J Vasc Interv Radiol* 16:1107–1119.
- Dahlin DC, Unni KK, eds. 1986. *Bone Tumors: General Aspects and Data on 8,542 Cases*, 4th ed. Springfield, IL: Charles C. Thomas.
- Delepine JW, Delepine G, Bacci G, Rosen G, Desbois JC. 1996. Influence of methotrexate dose intensity on outcome of patients with high grade osteogenic osteosarcoma. Analysis of the literature. *Cancer* 78:2127–2135.
- Demetri GD, Chawla SP, Ray-Coquard I, Le Cesne A, Staddon AP, Milhem MM, Penel N, Riedel RF, Bui-Nguyen B, Cranmer LD, Reichardt P, Bompas E, Alcindor T, Rushing D, Song Y, Lee RM, Ebbinghaus S, Eid JE, Loewy JW, Haluska FG, Dodion PF, Blay JY. 2013. Results of an international randomized phase III trial of the mammalian target of rapamycin inhibitor ridaforolimus versus placebo to control metastatic sarcomas in patients after benefit from prior chemotherapy. *J Clin Oncol* 31:2485–2492.
- Dow S, Elmslie R, Kurzman I, MacEwen G, Pericle F, Liggitt D. 2005. Phase I study of liposome-DNA complexes encoding the interleukin-2 gene in dogs with osteosarcoma lung metastases. *Hum Gene Ther* 16:937–946.
- Dubielzig RR, Biery DN, Brodey RS. 1981. Bone sarcomas associated with multifocal, medullary bone infarction in dogs. *J Am Vet Med Assoc* 179:64–68.
- Ehrhart NP. 2005. Longitudinal bone transport for treatment of primary bone tumors in dogs: Technique description and outcome in 9 dogs. *Vet Surg* 34:24–34.
- Ehrhart NP, Ryan SD, Fan TM. 2013. Tumors of the skeletal system. In: Withrow SJ, Vail DM, Page RL, eds. *Withrow & MacEwen's Small Animal Clinical Oncology*, 5th edition. St. Louis, MI: Saunders Elsevier. p 463–503.
- Eselgrim M, Grunert H, Kuhne T, Zoubek A, Kevric M, Bürger H, Jürgens H, Mayer-Steinacker R, Gosheger G, Bielack SS. 2006. Dose intensity of

- chemotherapy for osteosarcoma and outcome in the Cooperative Osteosarcoma Study Group (COSS) trials. *Pediatr Blood Cancer* 47:42–50.
- Fan TM, de Lorimier LP, Charney SC, Hintermeister JG. 2005. Evaluation of intravenous pamidronate administration in 33 cancer-bearing dogs with primary or secondary bone involvement. *J Vet Intern Med* 19:74–80.
- Fan TM, de Lorimier LP, O'Dell-Anderson K, Lacoste HI, Charney SC. 2007. Single-agent pamidronate for palliative therapy of canine appendicular osteosarcoma bone pain. *J Vet Intern Med* 21:431–439.
- Farese JP, Milner R, Thompson MS, Lester N, Cooke K, Fox L, Hester J, Bova FJ. 2004. Stereotactic radiosurgery for treatment of osteosarcomas involving the distal portions of the limbs in dogs. *J Am Vet Med Assoc* 225:1567–1572, 1548.
- Fieten H, Spee B, Ijzer J, Kik MJ, Penning LC, Kirpensteijn J. 2009. Expression of hepatocyte growth factor and the proto-oncogenic receptor c-Met in canine osteosarcoma. *Veterinary Pathology* 46:869–877.
- Flint AF, U'Ren L, Legare ME, Withrow SJ, Dernell W, Hanneman WH. 2004. Overexpression of the erbB-2 proto-oncogene in canine osteosarcoma cell lines and tumors. *Vet Pathol* 41:291–296.
- Forrest LJ, Dodge RK, Page RL, Heidner GL, McEntee MC, Novotny CA, Thrall DE. 1992. Relationship between quantitative tumor scintigraphy and time to metastasis in dogs with osteosarcoma. *J Nucl Med* 33:1542–1547.
- Fossey SL, Bear MD, Kisseberth WC, Pennell M, London CA. 2011. Oncostatin M promotes STAT3 activation, VEGF production, and invasion in osteosarcoma cell lines. *BMC Cancer* 11:125.
- Fossey SL, Bear MD, Lin J, Li C, Schwartz EB, Li PK, Fuchs JR, Fenger J, Kisseberth WC, London CA. 2011. The novel curcumin analog FLLL32 decreases STAT3 DNA binding activity and expression, and induces apoptosis in osteosarcoma cell lines. *BMC Cancer* 11:112.
- Fossey SL, Liao AT, McCleese JK, Bear MD, Lin J, Li PK, Kisseberth WC, London CA. 2009. Characterization of STAT3 activation and expression in canine and human osteosarcoma. *BMC Cancer* 9:81.
- Freeman SS, Allen SW, Ganti R, Wu J, Ma J, Su X, Neale G, Dome JS, Daw NC, Khoury JD. 2008. Copy number gains in EGFR and copy number losses in PTEN are common events in osteosarcoma tumors. *Cancer* 113:1453–1461.
- Gellasch KL, Kalscheur VL, Clayton MK, Muir P. 2002. Fatigue microdamage in the radial predilection site for osteosarcoma in dogs. *Am J Vet Res* 63:896–899.
- Gillette SM, Gillette EL, Powers BE, Withrow SJ. 1990. Radiation-induced osteosarcoma in dogs after external beam or intraoperative radiation therapy. *Cancer Res* 50:54–57.
- Gordon I, Paoloni M, Mazcko C, Khanna C. 2009. The Comparative Oncology Trials Consortium: Using spontaneously occurring cancers in dogs to inform the cancer drug development pathway. *PLoS Med* 6:e1000161.
- Gordon IK, Khanna C. 2010. Modeling opportunities in comparative oncology for drug development. *ILAR J* 51:214–220.
- Gordon IK, Ye F, Kent MS. 2008. Evaluation of the mammalian target of rapamycin pathway and the effect of rapamycin on target expression and cellular proliferation in osteosarcoma cells from dogs. *Am J Vet Res* 69:1079–1084.
- Gorlick R. 2009. Current concepts on the molecular biology of osteosarcoma. In: Jaffe N, Bruland OS, Bielack S, eds. *Pediatric and Adolescent Osteosarcoma, Cancer Treatment and Research*, Vol. 152. New York: Springer. p 467–478.
- Gorlick R, Khanna C. 2010. Osteosarcoma. *J Bone Miner Res* 25:683–691.
- Harris MB, Gieser P, Goorin AM, Ayala A, Shochat SJ, Ferguson WS, Holbrook T, Link MP. 1998. Treatment of metastatic osteosarcoma at diagnosis: A Pediatric Oncology Group Study. *J Clin Oncol* 16:3641–3648.
- Hong SH, Osborne T, Ren L, Briggs J, Mazcko C, Burkett SS, Khanna C. 2011. Protein kinase C regulates ezrin-radixin-moesin phosphorylation in canine osteosarcoma cells. *Vet Comp Oncol* 9:207–218.
- Jaroensong T, Endo Y, Lee SJ, Kamida A, Mochizuki M, Nishimura R, Sasaki N, Nakagawa T. 2012. Effects of transplantation sites on tumour growth, pulmonary metastasis and ezrin expression of canine osteosarcoma cell lines in nude mice. *Vet Comp Oncol* 10:274–282.
- Jeys LM, Grimer RJ, Carter SR, Tillman RM, Abudu A. 2007. Post-operative infection and increased survival in osteosarcoma patients: Are they associated? *Ann Surg Oncol* 14:2887–2895.
- Karlsson EK, Sigurdsson S, Ivansson E, Thomas R, Elvers I, Wright J, Howald C, Tonomura N, Perloski M, Swofford R, Biagi T, Fryc S, Anderson N, Courta-Cahen C, Youell L, Ricketts SL, Mandlebaum S, Rivera P, von Euler H, Kisseberth WC, London CA, Lander ES, Couto G, Comstock K, Starkey MP, Modiano JF, Breen M, Lindblad-Toh K. 2013. Genome-wide analyses implicate 33 loci in heritable dog osteosarcoma, including regulatory variants near CDKN2A/B. *Genome Biol* 14:R132.
- Kawai A, Sugihara S, Kunisada T, Hamada M, Inoue H. 1996. The importance of doxorubicin and methotrexate dose intensity in the chemotherapy of osteosarcoma. *Arch Orthop Trauma Surg* 115:68–70.
- Khanna C, Anderson PM, Hasz DE, Katsanis E, Neville M, Klausner JS. 1997. Interleukin-2 liposome inhalation therapy is safe and effective for dogs with spontaneous pulmonary metastases. *Cancer* 79:1409–1421.
- Khanna C, Lindblad-Toh K, Vail D, London C, Bergman P, Barber L, Breen M, Kitchell B, McNeil E, Modiano JF, Niemi S, Comstock KE, Ostrander E, Westmoreland S, Withrow S. 2006. The dog as a cancer model. *Nature Biotechnology* 24:1065–1066.
- Khanna C, Prehn J, Hayden D, Cassaday RD, Caylor J, Jacob S, Bose SM, Hong SH, Hewitt SM, Helman LJ. 2002. A randomized controlled trial of octreotide pamoate long-acting release and carboplatin versus carboplatin alone in dogs with naturally occurring osteosarcoma: Evaluation of insulin-like growth factor suppression and chemotherapy. *Clin Canc Res* 8:2406–2412.
- Khanna C, Wan X, Bose S, Cassaday R, Olomu O, Mendoza A, Yeung C, Gorlick R, Hewitt SM, Helman LJ. 2004. The membrane-cytoskeleton linker ezrin is necessary for osteosarcoma metastasis. *Nat Med* 10:182–186.
- Kirpensteijn J, Kik M, Rutteman GR, Teske E. 2002. Prognostic significance of a new histologic grading system for canine osteosarcoma. *Vet Pathol* 39:240–246.
- Kirpensteijn J, Kik M, Teske E, Rutteman GR. 2008. TP53 gene mutations in canine osteosarcoma. *Vet Surg* 37:454–460.
- Klein MJ, Siegal GP. 2006. Osteosarcoma: Anatomic and histologic variants. *Am J Clin Pathol* 125:555–581.
- Knecht CD, Priester WA. 1978. Musculoskeletal tumors in dogs. *J Am Vet Med Assoc* 172:72–74.
- Koshkina NV, Kleiner ES. 2005. Aerosol gemcitabine inhibits growth of primary osteosarcoma and osteosarcoma lung metastasis. *Int J Cancer* 116:458–463.
- Kuijjer ML, Peterse EF, van den Akker BE, Briaire-de Bruijn IH, Serra M, Meza-Zepeda LA, Myklebost O, Hassan AB, Hogendoorn PC, Cleton-Jansen AM. 2013. IR/IGF1R signaling as potential target for treatment of high-grade osteosarcoma. *BMC Cancer* 13:245.
- Kuntz CA, Asselin TL, Dernell WS, Powers BE, Straw RC, Withrow SJ. 1998. Limb salvage surgery for osteosarcoma of the proximal humerus: Outcome in 17 dogs. *Vet Surge* 27:417–422.
- Kurzman ID, MacEwen EG, Rosenthal RC, Fox LE, Keller ET, Helfand SC, Vail DM, Dubielzig RR, Madewell BR, Rodriguez CO Jr, Obradovich J, Fidel J, Rosenberg N. 1995. Adjuvant therapy for osteosarcoma in dogs: Results of randomized clinical trials using combined liposome-encapsulated muramyl tripeptide and cisplatin. *Clin Cancer Res* 1:1595–1601.
- LaRue SM, Withrow SJ, Powers BE, Wrigley RH, Gillette EL, Schwarz PD, Straw RC, Richter SL. 1989. Limb-sparing treatment for osteosarcoma in dogs. *J Am Vet Med Assoc* 195:1734–1744.
- Lascelles BDX, Dernell WS, Correa MT, Lafferty M, Devitt CM, Kuntz CA, Straw RC, Withrow SJ. 2005. Improved survival associated with postoperative wound infection in dogs treated with limb-salvage surgery for osteosarcoma. *Ann Surg Oncol* 12:1073–1083.
- Leibman NF, Kuntz CA, Steyn PF, Fettman MJ, Powers BE, Withrow SJ, Dernell WS. 2001. Accuracy of radiography, nuclear scintigraphy, and histopathology for determining the proximal extent of distal radius osteosarcoma in dogs. *Vet Surg* 30:240–245.
- Levine RA, Fleischli MA. 2000. Inactivation of p53 and retinoblastoma family pathways in canine osteosarcoma cell lines. *Vet Pathol* 37:54–61.

- Levine RA, Forest T, Smith C. 2002. Tumor suppressor PTEN is mutated in canine osteosarcoma cell lines and tumors. *Vet Pathol* 39:372–378.
- Lewis IJ, Nooij MA, Whelan J, Sydes MR, Grimer R, Hogendoorn PC, Memon MA, Weeden S, Uscinska BM, van Glabbeke M, Kirkpatrick A, Hauben EL, Craft AW, Taminiau AH, MRC B006 and EORTC 80931 collaborators; European Osteosarcoma Intergroup. 2007. Improvement in histologic response but not survival in osteosarcoma patients treated with intensified chemotherapy: A randomized phase III trial of the European Osteosarcoma Intergroup. *J Natl Cancer Inst* 99:112–128.
- Lewis IJ, Weeden S, Machin D, Stark D, Craft AW. 2000. Received dose and dose-intensity of chemotherapy and outcome in nonmetastatic extremity osteosarcoma. *European Osteosarcoma Intergroup. J Clin Oncol* 18:4028–4037.
- Liao AT, McCleese J, Kamerling S, Christensen J, London CA. 2007. A novel small molecule Met inhibitor, PF2362376, exhibits biological activity against osteosarcoma. *Vet Comp Oncol* 5:177–196.
- Liao AT, McMahon M, London CA. 2005. Characterization, expression and function of c-Met in canine spontaneous cancers. *Vet Comp Onc* 3:61–72.
- Liao AT, McMahon M, London CA. 2006. Identification of a novel germline MET mutation in dogs. *Anim Genet* 37:248–252.
- Linabery AM, Ross JA. 2008. Trends in childhood cancer incidence in the U.S. (1992–2004). *Cancer* 112:416–432.
- Lindblad-Toh K, Wade CM, Mikkelsen TS, Karlsson EK, Jaffe DB, Kamal M, Clamp M, Chang JL, Kulbokas EJ 3rd, Zody MC, Mauceli E, Xie X, Breen M, Wayne RK, Ostrander EA, Ponting CP, Galibert F, Smith DR, DeJong PJ, Kirkness E, Alvarez P, Biagi T, Brockman W, Butler J, Chin CW, Cook A, Cuff J, Daly MJ, DeCaprio D, Gnerre S, Grabherr M, Kellis M, Kleber M, Bardeleben C, Goodstadt L, Heger A, Hitte C, Kim L, Koepfli KP, Parker HG, Pollinger JP, Searle SM, Sutter NB, Thomas R, Webber C, Baldwin J, Abebe A, Abouelleil A, Aftuck L, Ait-Zahra M, Aldredge T, Allen N, An P, Anderson S, Antoine C, Arachchi H, Aslam A, Ayotte L, Bachantsang P, Barry A, Bayul T, Benamara M, Berlin A, Bessette D, Blitshteyn B, Bloom T, Blye J, Boguslavskiy L, Bonnet C, Boukhgalter B, Brown A, Cahill P, Calixte N, Camarata J, Cheshatsang Y, Chu J, Citroen M, Collymore A, Cooke P, Dawoe T, Daza R, Decktor K, DeGray S, Dhargay N, Dooley K, Dooley K, Dorje P, Dorjee K, Dorris L, Duffey N, Dupes A, Egbiremolen O, Elong R, Falk J, Farina A, Faro S, Ferguson D, Ferreira P, Fisher S, FitzGerald M, Foley K, Foley C, Franke A, Friedrich D, Gage D, Garber M, Gearin G, Giannoukos G, Goode T, Goyette A, Graham J, Grandbois E, Gyaltsen K, Hafez N, Hagopian D, Hagos B, Hall J, Healy C, Hegarty R, Honan T, Horn A, Houde N, Hughes L, Hunnicutt L, Husby M, Jester B, Jones C, Kamat A, Kanga B, Kells C, Khazanovich D, Kieu AC, Kisner P, Kumar M, Lance K, Landers T, Lara M, Lee W, Leger JP, Lennon N, Leuper L, LeVine S, Liu J, Liu X, Lokyitsang Y, Lokyitsang T, Lui A, Macdonald J, Major J, Marabella R, Maru K, Matthews C, McDonough S, Mehta T, Meldrim J, Melnikov A, Meneus L, Mihalev A, Mihova T, Miller K, Mittelman R, Mlenga V, Mulrain L, Munson G, Navidi A, Naylor J, Nguyen T, Nguyen N, Nguyen C, Nguyen T, Nicol R, Norbu N, Norbu C, Novod N, Nyima T, Olandt P, O'Neill B, O'Neill K, Osman S, Oyono L, Patti C, Perrin D, Phunxhang P, Pierre F, Priest M, Rachupka A, Raghuraman S, Rameau R, Ray V, Raymond C, Rege F, Rise C, Rogers J, Rogov P, Sahalie J, Settippalli S, Sharpe T, Shea T, Sheehan M, Sherpa N, Shi J, Shih D, Sloan J, Smith C, Sparrow T, Stalker J, Stange-Thomann N, Stavropoulos S, Stone C, Stone S, Sykes S, Tchuinga P, Tenzing P, Tesfaye S, Thoulutsang D, Thoulutsang Y, Topham K, Topping I, Tsamla T, Vassiliev H, Venkataraman V, Vo A, Wangchuk T, Wangdi T, Weiland M, Wilkinson J, Wilson A, Yadav S, Yang S, Yang X, Young G, Yu Q, Zainoun J, Zembek L, Zimmer A, Lander ES. 2005. Genome sequence, comparative analysis and haplotype structure of the domestic dog. *Nature* 438:803–819.
- Liptak JM, Dermell WS, Ehrhart N, Lafferty MH, Monteith GJ, Withrow SJ. 2006. Cortical allograft and endoprosthesis for limb-sparing surgery in dogs with distal radial osteosarcoma: A prospective clinical comparison of two different limb-sparing techniques. *Vet Surg* 35:518–533.
- Liptak JM, Dermell WS, Lascelles BD, Larue SM, Jameson VJ, Powers BE, Huber DJ, Withrow SJ. 2004. Intraoperative extracorporeal irradiation for limb sparing in 13 dogs. *Vet Surg* 33:446–456.
- London C, Mathie T, Stingle N, Clifford C, Haney S, Klein MK, Beaver L, Vickery K, Vail DM, Hershey B, Ettinger S, Vaughan A, Alvarez F, Hillman L, Kiselow M, Thamm D, Higginbotham ML, Gauthier M, Krick E, Phillips B, Ladue T, Jones P, Bryan J, Gill V, Novasad A, Fulton L, Carreras J, McNeill C, Henry C, Gillings S. 2012. Preliminary evidence for biologic activity of toceranib phosphate (Palladia) in solid tumors. *Vet Comp Oncol* 10:206–213.
- London CA, Bear MD, McCleese J, Foley KP, Paalangara R, Inoue T, Ying W, Barsoum J. 2011. Phase I evaluation of STA-1474, a prodrug of the novel HSP90 inhibitor ganetespib, in dogs with spontaneous cancer. *PLoS One* 6:e27018.
- Longhi A, Pasini A, Cicognani A, Baronio F, Pellacani A, Baldini N, Bacci G. 2005. Height as a risk factor for osteosarcoma. *J Pediatr Hematol Oncol* 27:314–318.
- Loukopoulos P, O'Brien T, Ghoddsi M, Mungall BA, Robinson WF. 2004. Characterization of three novel canine osteosarcoma cell lines producing high levels of matrix metalloproteinases. *Res Vet Sci* 77:131–141.
- Loukopoulos P, Thornton JR, Robinson WF. 2003. Clinical and pathologic relevance of p53 index in canine osseous tumors. *Vet Pathol* 40:237–248.
- Lucas PW, Fan TM, Garrett LD, Griffon DJ, Wypij JM. 2008. A comparison of five different bone resorption markers in osteosarcoma-bearing dogs, normal dogs, and dogs with orthopedic diseases. *J Vet Intern Med* 22:1008–1013.
- MacEwen EG, Kurzman ID, Rosenthal RC, Smith BW, Manley PA, Roush JK, Howard PE. 1989. Therapy for osteosarcoma in dogs with intravenous injection of liposome-encapsulated muramyl tripeptide. *J Natl Cancer Inst* 81:935–938.
- MacEwen EG, Kutzke J, Carew J, Pastor J, Schmidt JA, Tsan R, Thamm DH, Radinsky R. 2003. c-Met tyrosine kinase receptor expression and function in human and canine osteosarcoma cells. *Clin Exp Metastasis* 20:421–430.
- MacEwen EG, Pastor J, Kutzke J, Tsan R, Kurzman ID, Thamm DH, Wilson M, Radinsky R. 2004. IGF-1 receptor contributes to the malignant phenotype in human and canine osteosarcoma. *J Cell Biochem* 92:77–91.
- Machak GN, Tkachev SI, Solovyev YN, Sinyukov PA, Ivanov SM, Kochergina NV, Ryjkov AD, Tepliakov VV, Bokhian BY, Glebovskaya VV. 2003. Neoadjuvant chemotherapy and local radiotherapy for high-grade osteosarcoma of the extremities. *Mayo Clinic Proc* 78:147–155.
- Maeda J, Yurkon CR, Fujisawa H, Kaneko M, Genet SC, Roybal EJ, Rota GW, Saffer ER, Rose BJ, Hanneman WH, Thamm DH, Kato TA. 2012. Genomic instability and telomere fusion of canine osteosarcoma cells. *PLoS One* 7:e43355.
- Mangat KS, Jeys LM, Carter SR. 2011. Latest developments in limb-salvage surgery in osteosarcoma. *Expert Rev Anticancer Ther* 11:205–215.
- Maniscalco L, Iussich S, Morello E, Martano M, Biolatti B, Riondato F, Della Saldà L, Romanucci M, Malatesta D, Bongiovanni L, Tirrito F, Gattino F, Buracco P, De Maria R. 2013. PDGFs and PDGFRs in canine osteosarcoma: New targets for innovative therapeutic strategies in comparative oncology. *Vet J* 195:41–47.
- Marcellin-Little DJ, DeYoung DJ, Thrall DE, Merrill CL. 1999. Osteosarcoma at the site of bone infarction associated with total hip arthroplasty in a dog. *Vet Surg* 28:54–60.
- Mayr B, Eschborn U, Loupal G, Schleger W. 1991. Characterization of complex karyotype changes in two canine bone tumors. *Res Vet Sci* 51:341–343.
- McCleese JK, Bear MD, Fossey SL, Mihalek RM, Foley KP, Ying W, Barsoum J, London CA. 2009. The novel HSP90 inhibitor STA-1474 exhibits biologic activity against osteosarcoma cell lines. *Int J Cancer* 125:2792–2801.

- McCleese JK, Bear MD, Kulp SK, Mazcko C, Khanna C, London CA. 2013. Met interacts with EGFR and Ron in canine osteosarcoma. *Vet Comp Oncol* 11:124–139.
- McEntee MC, Page RL, Theon A, Erb HN, Thrall DE. 2004. Malignant tumor formation in dogs previously irradiated for acanthomatous epulis. *Vet Radiol Ultrasound* 45:357–361.
- McIntyre JF, Smith-Sorensen B, Friend SH, Jayne Kassell, Anne-Lise Borresen, Yu Xin Yan, Carolyn Russo, Judith Sato, Nodle Barbier, James Miser, David Malkin, Mark C Gebhardt. 1994. Germline mutations of the p53 tumor suppressor gene in children with osteosarcoma. *J Clin Oncol* 12:925–930.
- McMahon M, Mathie T, Stingle N, Romansik E, Vail D, London C. 2011. Adjuvant carboplatin and gemcitabine combination chemotherapy post-amputation in canine appendicular osteosarcoma. *J Vet Intern Med* 25:511–517.
- Mendoza S, Konishi T, Demell WS, Withrow SJ, Miller CW. 1998. Status of the p53, Rb and MDM2 genes in canine osteosarcoma. *Anticancer Res* 18:4449–4453.
- Meyers PA, Schwartz CL, Krailo MD, Healey JH, Bernstein ML, Betcher D, Ferguson WS, Gebhardt MC, Goorin AM, Harris M, Kleinerman E, Link MP, Nadel H, Nieder M, Siegal GP, Weiner MA, Wells RJ, Womer RB, Grier HE; Children's Oncology Group. 2008. Osteosarcoma: The addition of muramyl tripeptide to chemotherapy improves overall survival: A report from the Children's Oncology Group. *J Clin Oncol* 26:633–638.
- Meyers PA, Schwartz CL, Krailo M, Kleinerman ES, Betcher D, Bernstein ML, Conrad E, Ferguson W, Gebhardt M, Goorin AM, Harris MB, Healey J, Huvos A, Link M, Montebello J, Nadel H, Nieder M, Sato J, Siegal G, Weiner M, Wells R, Wold L, Womer R, Grier H. 2005. Osteosarcoma: A randomized, prospective trial of the addition of ifosfamide and/or muramyl tripeptide to cisplatin, doxorubicin, and high-dose methotrexate. *J Clin Oncol* 23:2004–2011.
- Miller SC, Loyd RD, Bruenger FW, Krahenbuhl MP, Polig E, Romanov SA. 2003. Comparisons of the skeletal locations of putative plutonium-induced osteosarcoma in humans with those in beagle dogs and with naturally tumors in both species. *Radiat Res* 160:517–523.
- Mintz MB, Sowers R, Brown KM, Hilmer SC, Mazza B, Huvos AG, Meyers PA, Lafleur B, McDonough WS, Henry MM, Ramsey KE, Antonescu CR, Chen W, Healey JH, Daluski A, Berens ME, Macdonald TJ, Gorlick R, Stephan DA. 2005. An expression signature classifies chemotherapy-resistant pediatric osteosarcoma. *Cancer Res* 65:1748–1754.
- Mirabello L, Troisi RJ, Savage SA. 2009. Osteosarcoma incidence and survival rates from 1973 to 2004: Data from the Surveillance, Epidemiology, and End Results Program. *Cancer* 115:1531–1543.
- Misdorp W, Hart AA. 1979. Some prognostic and epidemiologic factors in canine osteosarcoma. *J Natl Cancer Inst* 62:537–545.
- Mitchell L, Thamm DH, Biller BJ. 2012. Clinical and immunomodulatory effects of toceranib combined with low-dose cyclophosphamide in dogs with cancer. *J Vet Intern Med* 26:355–362.
- Modiano JF, Bellgrau D, Cutter GR, Lana SE, Ehrhart NP, Ehrhart E, Wilke VL, Charles JB, Munson S, Scott MC, Pozniak J, Carlson CS, Schaack J, Duke RC. 2012. Inflammation, apoptosis, and necrosis induced by neoadjuvant fas ligand gene therapy improves survival of dogs with spontaneous bone cancer. *Mol Ther* 20:2234–2243.
- Mohseny AB, Hogendoorn PCW, Cleton-Jansen AM. 2012. Osteosarcoma models: From cell lines to zebrafish. *Sarcoma* 2012:417271.
- Moore AS, Dernel WS, Ogilvie GK, Kristal O, Elmslie R, Kitchell B, Susaneck S, Rosenthal R, Klein MK, Obradovich J, Legendre A, Haddad T, Hahn K, Powers BE, Warren D. 2007. Doxorubicin and BAY 12–9566 for the treatment of osteosarcoma in dogs: A randomized, double-blind, placebo-controlled study. *J Vet Intern Med* 21:783–790.
- Morello E, Vasconi E, Martano M, Peirone B, Buracco P. 2003. Pasteurized tumoral autograft and adjuvant chemotherapy for the treatment of canine distal radial osteosarcoma: 13 cases. *Vet Surg* 32:539–544.
- O'Donoghue LE, Pritsyn AA, Kamstock DA, Siebert J, Thomas RS, Duval DL. 2010. Expression profiling in canine osteosarcoma: Identification of biomarkers and pathways associated with outcome. *BMC Cancer* 10:506.
- Onimoe GI, Liu A, Lin L, Wei CC, Schwartz EB, Bhasin D, Li C, Fuchs JR, Li PK, Houghton P, Termuhlen A, Gross T, Lin J. 2012. Small molecules, LLL12 and FLLL32, inhibit STAT3 and exhibit potent growth suppressive activity in osteosarcoma cells and tumor growth in mice. *Invest New Drugs* 30:916–926.
- Ottaviani G, Jaffe N. 2009. The epidemiology of osteosarcoma. The etiology of osteosarcoma. In: Jaffe N, Bruland OS, Bielack S, eds. *Pediatric and Adolescent Osteosarcoma, Cancer Treatment and Research*, 152nd vol. New York: Springer. p 3–32.
- Oya N, Kokubo M, Mizowaki T, Shibamoto Y, Nagata Y, Sasai K, Nishimura Y, Tsuboyama T, Toguchida J, Nakamura T, Hiraoka M. 2001. Definitive intraoperative very high-dose radiotherapy for localized osteosarcoma in the extremities. *Int J Radiat Oncol Biol Phys* 51:87–93.
- Paoloni M, Davis S, Lana S, Withrow S, Sangiorgi L, Picci P, Hewitt S, Triche T, Meltzer P, Khanna C. 2009. Canine tumor cross-species genomics uncovers targets linked to osteosarcoma progression. *BMC Genomics* 10:625.
- Paoloni M, Khanna C. 2008. Translation of new cancer treatments from pet dogs to humans. *Nat Rev Cancer* 8:147–156.
- Paoloni MC, Mazcko C, Fox E, Fan T, Lana S, Kisseberth W, Vail DM, Nuckolls K, Osborne T, Yalkowsy S, Gustafson D, Yu Y, Cao L, Khanna C. 2010. Rapamycin pharmacokinetic and pharmacodynamic relationships in osteosarcoma: A comparative oncology study in dogs. *PLoS One* 5:e11013.
- Patanè S, Avnet S, Coltella N, Costa B, Sponza S, Olivero M, Vigna E, Naldini L, Baldini N, Ferracini R, Corso S, Giordano S, Comoglio PM, Di Renzo MF. 2006. MET overexpression turns human primary osteoblasts into osteosarcomas. *Cancer Res* 66:4750–4757.
- Powers BE, Gillette EL, McChesney SL, LeCouteur RA, Withrow SJ. 1989. Bone necrosis and tumor induction following experimental intraoperative irradiation. *Int J Radiat Oncol Biol Phys* 17:559–567.
- Provisor AJ, Ettinger LJ, Nachman JB, Krailo MD, Makley JT, Yunis EJ, Huvos AG, Betcher DL, Baum ES, Kisker CT, Miser JS. 1997. Treatment of nonmetastatic osteosarcoma of the extremity with preoperative and postoperative chemotherapy: A report from the Children's Cancer Group. *J Clin Oncol* 15:76–84.
- Raffaella De M, Silvia M, Selina I, Martina O, Emanuela M, Andrea B, James GC, Bartolomeo B, Roy AL, Paolo B, Maria Flavia Di R. 2009. Met oncogene activation qualifies spontaneous canine osteosarcoma as a suitable pre-clinical model of human osteosarcoma. *J Pathol* 218:399–408.
- Rainusso N, Wang LL, Yustein JT. 2013. The adolescent and young adult with cancer: State of the art: Bone tumors. *Curr Oncol Rep* 15:296–307.
- Richards KL, Motsinger-Reif AA, Chen HW, Fedoriw Y, Fan C, Nielsen DM, Small GW, Thomas R, Smith C, Dave SS, Perou CM, Breen M, Borst LB, Suter SE. 2013. Gene profiling of canine B-cell lymphoma reveals germinal center and postgerminal center subtypes with different survival times, modeling human DLBCL. *Cancer Res* 73:5029–5039.
- Riser WH, Brodey RS, Biery DN. 1972. Bone infarctions associated with malignant bone tumors in dogs. *J Am Vet Med Assoc* 160:414–421.
- Rissetto KC, Rindt H, Selting KA, Villamil JA, Henry CJ, Reinero CR. 2010. Cloning and expression of canine CD25 for validation of an anti-human CD25 antibody to compare T regulatory lymphocytes in healthy dogs and dogs with osteosarcoma. *Vet Immunol Immunopathol* 135:137–145.
- Rodriguez CO Jr, Crabbs TA, Wilson DW, Cannan VA, Skorupski KA, Gordon N, Koshkina N, Kleinerman E, Anderson PM. 2010. Aerosol gemcitabine: Preclinical safety and in vivo antitumor activity in osteosarcoma-bearing dogs. *J Aerosol Med Pulm Drug Deliv* 23:197–206.
- Rowell JE, McCarthy DO, Alvarez CE. 2011. Dog models of naturally occurring cancer. *Trends Mol Med* 17:380–388.
- Ru G, Terracini B, Glickman LT. 1998. Host related risk factors for canine osteosarcoma. *Vet J* 156:31–39.
- Rusk A, McKeegan E, Haviv F, Majest S, Henkin J, Khanna C. 2006. Preclinical evaluation of antiangiogenic thrombospondin-1 peptide

- mimetics, ABT-526 and ABT-510, in companion dogs with naturally occurring cancers. *Clin Cancer Res*. 2006 Dec 15;12:7444–7455.
- Ryan SD, Timbie JW, Haussler KK, et al. 2011. Randomized, placebo-controlled, clinical trial of radiation therapy with or without pamidronate for palliative treatment of canine appendicular osteosarcoma. *Vet Comp Oncol* 9:e1–e49.
- Ryu K, Choy E, Yang C, Susa M, Hornicek FJ, Mankin H, Duan Z. 2010. Activation of signal transducer and activator of transcription 3 (Stat3) pathway in osteosarcoma cells and overexpression of phosphorylated-Stat3 correlates with poor prognosis. *J Orthop Res* 28:971–978.
- Sarver AL, Thayanithy V, Scott MC, Cleton-Jansen AM, Hogendoorn PC, Modiano JF, Subramanian S. 2013. MicroRNAs at the human 14q32 locus have prognostic significance in osteosarcoma. *Orphanet J Rare Dis* 8:7.
- Schmidt AF, Nielsen M, Klungel OH, Hoes AW, de Boer A, Groenwold RH, Kirpensteijn J. 2013. Prognostic factors of early metastasis and mortality in dogs with appendicular osteosarcoma after receiving surgery: An individual patient data meta-analysis. *Prev Vet Med* 112:414–422.
- Scott MC, Sarver AL, Gavin KJ, Thayanithy V, Getzy DM, Newman RA, Cutter GR, Lindblad-Toh K, Kisseberth WC, Hunter LE, Subramanian S, Breen M, Modiano JF. 2011. Molecular subtypes of osteosarcoma identified by reducing tumor heterogeneity through an interspecies comparative approach. *Bone* 49:356–367.
- Sequin B, Walsh PJ, Mason DR, Wisner ER, Parmenter JL, Dernel WS. 2003. Use of an ipsilateral vascularized ulnar transposition autograft for limb-sparing surgery of the distal radius in dogs: An anatomic and clinical study. *Vet Surg* 32:69–79.
- Selmic LE, Burton JH, Thamm DH, Withrow SJ, Lana SE. 2014. Comparison of carboplatin and doxorubicin-based chemotherapy protocols in 470 dogs after amputation for treatment of appendicular osteosarcoma. *J Vet Intern Med Epub Feb* 10.
- Selvarajah GT, Kirpensteijn J, van Wolferen ME, Rao NA, Fieten H, Mol JA. 2009. Gene expression profiling of canine osteosarcoma reveals genes associated with short and long survival times. *Mol Cancer* 8:72.
- Selvarajah S, Yoshimoto M, Ludkovski O, Park PC, Bayani J, Thorner P, Maire G, Squire JA, Zielenska M. 2008. Genomic signatures of chromosomal instability and osteosarcoma progression detected by high resolution array CGH and interphase FISH. *Cytogenet Genome Res* 122:5–15.
- Sherger M, Kisseberth W, London C, Olivo-Marston S, Papenfuss TL. 2012. Identification of myeloid derived suppressor cells in the peripheral blood of tumor bearing dogs. *BMC Vet Res* 8:209.
- Sottnik JL, Duval DL, Ehrhart EJ, Thamm DH. 2010. An orthotopic, post-surgical model of luciferase transfected murine osteosarcoma with spontaneous metastasis. *Clin Exp Metastasis* 27:151–160.
- Sottnik JL, Rao S, Lafferty MH, Thamm DH, Morley PS, Withrow SJ, Dow SW. 2010. Association of blood monocyte and lymphocyte count and disease-free interval in dogs with osteosarcoma. *J Vet Intern Med* 24:1439–1444.
- Sottnik JL, U'Ren LW, Thamm DH, Withrow SJ, Dow SW. 2010. Chronic bacterial osteomyelitis suppression of tumor growth requires innate immune responses. *Cancer Immunol Immunother* 59:367–378.
- Spodnick GJ, Berg J, Rand WM, Shelling SH, Couto G, Harvey HJ, Henderson RA, MacEwen G, Mauldin N, McCaw DL. 1992. Prognosis for dogs with appendicular osteosarcoma treated with amputation alone: 162 cases (1978–1988). *J Am Vet Med Assoc* 200:995–999.
- Stevenson S, Holm RB, Pohler OE, Fetter AW, Olmstead ML, Wind AP. 1982. Fracture-associated sarcoma in the dog. *J Am Vet Med Assoc* 180:1189–1196.
- Stevenson S, Shaffer JW, Goldberg VM. 1996. The humoral response to vascular and nonvascular allografts of bone. *Clin Orthop Relat Res* 326:86–95.
- Straw RC, Powers BE, Withrow SJ, Cooper MF, Turner AS. 1992. The effect of intramedullary polymethylmethacrylate on healing of intercalary cortical allografts in a canine model. *J Ortho Res* 10:434–439.
- Thomas R, Duke SE, Bloom SK, Breen TE, Young AC, Feiste E, Seiser EL, Tsai PC, Langford CF, Ellis P, Karlsson EK, Lindblad-Toh K, Breen M. 2007. A cytogenetically characterized, genome-anchored 10-Mb BAC set and CGH array for the domestic dog. *J Hered* 98:474–484.
- Thomas R, Wang HJ, Tsai PC, Langford CF, Fosmire SP, Jubala CM, Getzy DM, Cutter GR, Modiano JF, Breen M. 2009. Influence of genetic background on tumor karyotypes: Evidence for breed-associated cytogenetic aberrations in canine appendicular osteosarcoma. *Chrom Res* 17:365–377.
- Thrall DE, Withrow SJ, Powers BE, Straw RC, Page RL, Heidner GL, Richardson DC, Bissonnette KW, Betts CW, DeYoung DJ, et al. 1990. Radiotherapy prior to cortical allograft limb sparing in dogs with osteosarcoma: A dose response assay. *Int J Radiat Oncol Biol Phys* 18:1351–1357.
- Tommasini M, Ehrhart N, Ferretti A, Buracco P. 2000. Bone transport osteogenesis for limb salvage following resection of primary bone tumors: Experience with 6 cases (1991–1996). *Vet Comp Orthop Traumatol* 23:43–51.
- Walkley CR, Qudsi R, Sankaran VG, Perry JA, Gostissa M, Roth SI, Rodda SJ, Snay E, Dunning P, Fahey FH, Alt FW, McMahon AP, Orkin SH. 2008. Conditional mouse osteosarcoma, dependent on p53 loss and potentiated by loss of Rb, mimics the human disease. *Genes Dev* 22:1662–1676.
- Walter CU, Dernel WS, LaRue SM, Lana SE, Lafferty MH, LaDue TA, Withrow SJ. 2005. Curative-intent radiation therapy as a treatment modality for appendicular and axial osteosarcoma: A preliminary retrospective evaluation of 14 dogs with the disease. *Vet Comp Oncol* 3:1–7.
- Wang YC, Zheng LH, Ma BA, Zhou Y, Zhang MH, Zhang DZ, Fan QY. 2011. Clinical value of signal transducers and activators of transcription 3 (STAT3) gene expression in human osteosarcoma. *Acta Histochem* 113:402–408.
- Wang YH, Han XD, Qiu Y, Xiong J, Yu Y, Wang B, Zhu ZZ, Qian BP, Chen YX, Wang SF, Shi HF, Sun X. 2012. Increased expression of insulin-like growth factor-1 receptor is correlated with tumor metastasis and prognosis in patients with osteosarcoma. *J Surg Oncol* 105:235–243.
- Wang YH, Wang ZX, Qiu Y, Xiong J, Chen YX, Miao DS, De W. 2009. Lentivirus-mediated RNAi knockdown of insulin-like growth factor-1 receptor inhibits growth, reduces invasion, and enhances radiosensitivity in human osteosarcoma cells. *Mol Cell Biochem* 327:257–266.
- Wilkins RM, Cullen JW, Camozzi AB, Jamroz BA, Odom L. 2005. Improved survival in primary nonmetastatic pediatric osteosarcoma of the extremity. *Clin Ortho Rel Res* 438:128–136.
- Wilson H, Heulsmeyer M, Chun R, Young KM, Friedrichs K, Argyle DJ. 2008. Isolation and characterization of cancer stem cells from canine osteosarcoma. *Vet J* 175:69–75.
- Withrow SF, Powers BE, Straw RC, Wildins RM. 1991. Comparative aspects of osteosarcoma. Dog versus man. *Clin Orthop Relat Res* 270:159–168.
- Withrow SF, Wilkins RM. 2010. Cross talk from pets to people: Translational osteosarcoma treatments. *ILAR* 51:208–213.
- Withrow SJ, Thrall DE, Straw RC, Powers BE, Wrigley RH, Larue SM, Page RL, Richardson DC, Bissonnette KW. 1993. Intra-arterial cisplatin with or without radiation in limb-sparing for canine osteosarcoma. *Cancer* 71:2484–2490.
- Wunder JS, Gokgoz N, Parkes R, Bull SB, Eskandarian S, Davis AM, Beauchamp CP, Conrad EU, Grimer RJ, Healey JH, Malkin D, Mangham DC, Rock MJ, Bell RS, Andriulis IL. 2005. TP53 mutations and outcome in osteosarcoma: A prospective, multicenter study. *J Clin Oncol* 23:1483–1490.
- Xiao H, Chen L, Luo G, Son H, Prectoni JH, Zheng W. 2013. Effect of the cytokine levels in serum on osteosarcoma. *Tumour Biol* 35:1023–1028.