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Review

Comparative Biology of Human and Canine Osteosarcoma

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Abstract. *Dogs with osteosarcoma provide an important model for the same disease in humans. In this report, the comparative nature of human and canine osteosarcoma including incidence and risk factors, clinical presentation and diagnosis, genetic abnormalities, biologic behaviour and prognostic factors, as well as treatment options are reviewed.*

Naturally occurring tumours in dogs provide an important model of human cancer biology and cancer therapeutic strategies (1, 2). Several features make dogs attractive models for human cancer. First, there is greater genetic homology between dogs and humans than between either species and the mouse (3, 4). Second, companion animals live in the same environment as humans and share the similar environmental risk factors. Dogs represent a more outbred population than inbred laboratory animals. Spontaneously developing tumours in dogs share many aspects in tumour biology and behaviour, whereas tumours in experimental laboratory animals are usually induced artificially. Body size and cell kinetics in dogs are comparable to those of humans and sample collection, surgical intervention and imaging is more rapidly applied than in rodent models.

Cancer is one of the main causes of death from disease both in humans and dogs, so a large population of animals with cancer can be included in studies. Furthermore, the incidence of many malignancies in dogs is higher than in humans and the progression is usually faster, which allows rapid accrual of data. The lack of "gold standard" treatment regimens in veterinary cancer patients allows more latitude in prospective clinical trials and makes it easier to investigate novel treatment strategies. Research costs for clinical trials using veterinary patients are significantly less than these for human clinical trials.

Until recently, a significant weakness of studying cancer biology in canine cancer models has been the relative lack of species-specific investigational tools, *e.g.,* molecular reagents, monoclonal antibodies, DNA probes and libraries, and canine recombinant products. However, molecular technologies are advancing and efforts to validate reagents and further characterize canine models have been ongoing and show great promise (5).

For each canine cancer model, there are important similarities with and differences from the human disease, and understanding of both is necessary for the appropriate use of dogs as models for human disease. The canine malignancies that offer the best comparative studies include osteosarcoma (6), lymphoma/leukaemia, soft tissue sarcoma, melanoma and mammary tumours (1, 7). The purpose of this report is to review the comparative nature of canine and human osteosarcoma and the current trends in clinical research using dogs as models for human osteosarcoma.

Incidence and Risk Factors

Osteosarcoma is a high-grade primary bone neoplasm of mesenchymal origin. Worldwide, the incidence of osteosarcoma in humans has been reported to be in the range of 1 to 3 cases per million annually (8). Osteosarcoma can occur as secondary tumour originating at the anatomic site of a pre-existing but otherwise unrelated pathological condition such as Paget disease. Rarely, osteosarcoma can occur extraskeletally, and most often, these patients are older than 30 years of age. The majority of patients diagnosed with osteosarcoma are in their second decade of life. Approximately 70% to 75% of cases occur between the ages 10 and 25 years. There is an overall male predominance in gender predilection, with a male:female ratio of 1.5:1. The lesion is most often found in the metaphysis of long bones. Approximately 50% of the lesions occur around the knee (9).The femur is the single most common anatomic location (45%), followed by the tibia

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(15%) and the humerus (10%). The precise etiology of osteosarcoma is unknown. However, there are genetic predispositions, such as the Li-Fraumeni, the Rothmund-Thomson and the Bloom syndromes. Several chemical agents, such as beryllium, and the FBJ-virus were shown to be inducers of osteosarcoma. Irradiation has been shown to induce secondary osteosarcoma and even electrical burn and trauma are thought to be other contributing factors of osteosarcoma pathogenesis (10).

Osteosarcoma is the most common primary bone tumour in dogs, accounting for up to 85% of malignancies originating in the skeleton (11). It is estimated to occur in over 8,000 dogs each year in the United States (6, 12); the actual number is probably higher, since not all cases are confirmed nor registered. Osteosarcoma tends to occur in middle-aged to older dogs, with a median age of 7 years, with some reports showing a bimodal age distribution with a second small peak between 18 and 24 months (13). Approximately 75% of osteosarcoma occur in the appendicular skeleton (14, 15). As in humans, the metaphyseal region of long bones is the most common primary site of osteosarcoma, with front limbs affected twice as often as rear limbs and the distal radius (35%) and proximal humerus (18%) being the two most common locations (16). Osteosarcoma is classically a cancer of large and giant breeds (17). Only 5% of osteosarcoma occur in dogs weighing less than 15 kg, and most of their tumours originate in the axial skeleton (18). Extraskeletal osteosarcoma is a rare neoplasm in older dogs (median age 11 years) that appears to carry a worse prognosis than skeletal osteosarcoma (19-21). The etiology of canine osteosarcoma is generally unknown. Mutagenic effects of ionizing radiation (22-24), multiple minor trauma (*e.g.,* metallic implants) (25-27) and genetic alterations (28-39) are possible risk factors.

Clinical Presentation and Diagnosis

Most human patients diagnosed with osteosarcoma report a brief history of pain and swelling of the involved limb. The pain may be intermittent at first, only later become constant, particularly at night. Due to the age of these patients, pain around the knee is often mistaken as a sports injury and is not recognized until the tumour progresses in size. Hematogenic micrometastasis occur most often in the lungs and develop in approximately half of all patients.

Dogs with appendicular osteosarcoma usually present with acute or chronic onset of lameness and/or swelling of the affected limb. The diagnostic work-up of the osteosarcoma of dogs is the same as for humans. Regional radiographs of the affected area most commonly reveal aggressive, metaphyseal lesions of the long bones. The radiographic appearance of osteosarcoma can range from lytic to blastic and is usually a mixture of both (40). The gold standard for diagnosis is considered to be biopsy with histopathological evaluation (41), although fine-needle aspiration and cytology of lytic lesions is gradually being used more frequently in veterinary medicine (42). Histologically, osteosarcoma is described as a malignant spindle cell tumour characterized by the production of an osteoid matrix by the tumour cells. In addition, mainly human patients undergo whole body bone scans, CT and MRI to precisely define the local extent of the tumour, as well as CT or, in selected cases, PET-CT of the lung to assess metastatic disease.

Genetic Abnormalities

Tumour suppressor pathways. Recent data suggest that specific pathways may play an important role in the pathogenesis of osteogenic sarcoma. *p53* and *Rb* alterations are frequent events and thought to contribute to the initiation of the tumour. Further, *RECQ* helicases, as well as telomere maintenance, are crucial for early progression (10, 43). The *P53* protein acts as a major regulator of cell replication and *p53* mutations lead to un-regulated replicative capability of the affected cell. *MDM2* is an important regulator of *p53*. In human osteosarcoma, the overall frequency of *p53* mutations ranges from 15% to 30% (44). The canine *p53* has been localized on chromosome 5 (45), the complete *p53* wild-type cDNA has been isolated and sequenced (46, 47) and the full length of the wild-type canine p53 protein has been characterized (47). A strong homology exists, with the conserved domains II, III, IV, and V being identical in humans and dogs (48). Alterations in the *p53* gene have been demonstrated in a number of canine tumours, including osteosarcoma. Mutations were found, depending on the study, in 7 of 15 appendicular osteosarcoma (28), 4 of 17 (39), and 8 of 21 (32) primary osteosarcoma screened, as well as in osteosarcoma cell lines (31), so both human and canine osteosarcoma patients have *p53* mutations at similar frequencies. However, one of the unique mutations affecting the *p53* gene in human osteosarcoma were large deletions or rearrangements. No such gross gene alterations could be identified in canine osteosarcoma, which suggests a different mutagenic effector (32).

Human osteosarcoma often harbour mutations in the *Rb* pathway, and it is thought that 60% of patients or more have *Rb*-associated abnormalities (49). Osteosarcoma tumourigenesis in humans results if both alleles at the retinoblastoma susceptibility locus (*Rb* gene) are altered. *Rb* gene alterations may be pertinent to the genesis of most human osteosarcoma cases and some other bone and soft tissue tumours (50). Normally, *Rb* protein blocks cell proliferation by controlling the availability and activity of specific members of the E2F family of transcription factors that govern the progression from G1- to the S-phase of the

cell cycle (51). Ultimately, if the *pRb*-pathway is disrupted, the cell becomes insensitive to antigrowth factors that block the advancement to the G1-phase, which results in uncontrolled cell proliferation. The role of *Rb* gene abnormalities in the tumourigenesis of canine osteosarcoma might not be as important as in the human disease (32). Instead, canine osteosarcoma cell lines contain mutations that indirectly inactivate the three *Rb* family members, *Rb*, *p107* and *p130,* simultaneously (31).

Another important tumour suppressor gene, *PTEN*, plays an important role in regulating cell proliferation, migration, and survival, as well as tumour cell invasion and tumour-directed angiogenesis (52-56). *RECQ* helicases are DNA unwinding enzymes that are involved in many basic cellular processes, including DNA replication, transcription, and repair and are believed to play a role in tumour suppression. Mutations in the *RECQL4* gene have been identified in approximately two thirds of patients with Rothmund-Thomson syndrome, and the presence of mutations is correlated with the risk of developing osteosarcoma (57). *RECQ* have not been evaluated in canine osteosarcoma. *PTEN* is mutated in a wide range of human tumours (58), but this has not been evaluated in osteosarcomas. *PTEN* was investigated in canine osteosarcoma cell lines (30) and four of five of these cell lines expressed high levels of the phosphorylated form of Akt, which may be an indirect indicator of aberrant *PTEN* expression.

Oncogene overexpression. Although many proto-oncogenes are overexpressed in osteosarcomas, the exact role of this overepression in the pathogenesis of osteosarcoma is not yet clear. *erbB-2* is a proto-oncogene that encodes human epidermal growth factor receptor 2 (*HER-2*), which, when activated, induces a cascade of mechanisms resulting in cell transformation and growth (59). The role of *Her2* in human is controversial, overexpression of *HER-2*/*erbB2* in human osteosarcoma has been shown by several groups, who suggest a correlation with poor clinical outcome (60-62), whereas other groups have found overexpression to be very rare or absent (63-66). The expression of *erbB-2* has been evaluated in seven canine osteosarcoma cell lines and 10 canine osteosarcoma tissue samples (67), and was found to be overexpressed in 86% of the cell lines and 40% of the osteosarcoma tissue samples.

Other oncogenes that have been investigated in human osteosarcoma include *c-myc, c-fos, MET, SAS*, and *GLI*. Both *c-myc* and *c-fos* have been shown to be overexpressed in human osteosarcoma (68, 69). A study evaluating the proto-oncogenes *c-sis, c-myc, N-myc,* and *cH-ras* in 9 canine osteosarcoma and 17 normal canine tissue samples revealed significant amplifications of the *c-sis* and *c-myc* genes in tumour tissue (36). This study also showed similar levels of expression of the sis gene product, *PDGF-β*, in human and in canine osteosarcoma by immunostaining. Another group described an overexpression of the sis oncogene in a canine osteosarcoma cell line, and all the canine osteosarcoma cell lines tested in that study contained PDGF receptors (29). This suggests the possibility of an autocrine growth factor loop participating in the pathogenesis of a subset of canine osteosarcomas.

Insulin-linke growth factor-1 (*IGF-1*) and *IGF* binding proteins (*IGFBPs*) have major regulatory function on the physiology of osteoblasts (70). Osteosarcoma cells have been shown to express *IGF-I* and *IGF-I* receptors, proliferate in response to *IGF-I* and demonstrate an antiapoptotic phenotype *in vitro* (71, 72). Human preclinical studies suggest a role of *IGF-1* in the proliferation of osteosarcoma cells *in vivo* (73), although so far, studies have failed to demonstrate a correlation between *IGF-1* or *IGF-1R* expression with clinical behaviour of human osteosarcoma (74, 75). Only one study revealed that high IGF expression was correlated with a lower apoptotic rate of osteosarcoma cells following chemotherapy (76). In a preclinical study in pet dogs, the suppression of serum *IGF* levels using a long-acting analogue of somatostatin (OncoLAR) did not improve chemotherapy related antitumour effects (77). Recently, *IGF-1* was shown to play a significant role on osteosarcoma cell growth and invasion in canine and human osteosarcoma cell lines (34).

Another study characterized the role of hepatocyte growth factor (*HGF*) and its receptor *c-Met* in human osteosarcoma cell lines and cells derived from spontaneous canine osteosarcoma (35). Their results indicate that *c-Met* and *HGF-SG* may contribute to the malignant phenotype of human and canine osteosarcoma as well. A study investigating the Met receptor activation in five human osteosarcoma cell lines revealed that *HGF* activates both mitogen and motogen machineries in these osteosarcoma cells (78). A variety of different canine tumour cell lines including osteosarcma cell lines were screened for the expression of Met, *HGF* and *HGFA* in a recent study (79). Overexpression of *Met* was present in all cell lines.

Metastasis

Human osteosarcoma most often metastasizes to the lung and bone. Lymph node metastasis is rare. At diagnosis, approximately 80% of patients are believed to have micro-metastatic disease, but they are only detectable in 8-15% with current diagnostic tools (80, 81). For these reasons, many patients have undetectable pulmonary micrometastasis at diagnosis. The 5-year survival rate is between 65% and 75% for localized disease, whereas for patients with metastasis at presentation, it remains poor at 20% (82, 83). Despite the advances in surgical and medical management, the mechanisms underlying osteosarcoma progression and metastasis is still to be elucidated.

In dogs, osteosarcoma has very aggressive local effects and metastases are very common. The pulmonary parenchyma is the most common metastatic site (13, 14). In a retrospective study, histologically confirmed regional lymph node metastases were found in 4.4% of 228 dogs with osteosarcoma at the time of limb amputation (84). An increase in the incidence of bone and soft tissue metastasis following chemotherapy has been documented in both humans and in dogs (85-90). Although less than 15% of dogs have radiographically detectable pulmonary metastases at initial evaluation, occult metastatic disease is present in approximately 90% of dogs at presentation (11). The usual cause of death in humans and in dogs following amputation as the sole treatment for osteosarcoma is diffuse pulmonary metastasis (91).

Metastasis-related genes. Gene expression profiling through the use of complementary DNA microarray analysis is beginning to uncover the molecular events that dictate the metastatic potential. These findings may pave the way for future molecularly targeted therapies. In humans, a few such markers have been indentified: ezrin, a membrane-cytoskeleton linker, a member of the ERM family of proteins (92), Annexin-2, which belongs to a family of diverse proteins characterized by by conserved annexin repeat domains and the ability to bind negatively-charged phospholipids in a calcium-dependent manner (93); CXCR4 or chemokine receptor-4, which belongs to a family of cytokine-like proteins that play a role in cytoskeleton rearrangement, adhesion to endothelial cells, and directional migration (94, 95), and Fas-ligand and its receptor, a member of the tumour necrosis factor receptor family and a transmembrane protein that induces apoptosis in susceptible cells by interacting with its receptor.

Ezrin protein was detected in 83% of primary osteosarcoma in dogs (92). The presence of high ezrin staining in the primary tumour was associated with a significantly shorter median disease-free interval (DFI; 116 d) compared to dogs with low ezrin staining (median DFI 188 d (92)). A similar correlation between ezrin staining and DFI and survival was found in a small set of prospectively collected tissues from pediatric human patients with osteosarcoma, where ezrin was expressed in 92% of the samples. In one study, rapamycin was shown to inhibit ezrin-mediated metastatic behaviour in a murine model of osteoarcoma by blocking the mTOR/S6K1/4E-BP-1 pathway (96). These results suggest that blocking this pathway may be an appropriate target for strategies to reduce tumour cell metastasis.

Prognostic Factors

In humans, the single most important prognostic parameters are the development of metastasis and unresponsiveness to chemotherapy, or both. It has been shown that patients who presented with metastasis at diagnosis have a worse prognosis than those with whom metastasis is diagnosed during or subsequent to completion of chemotherapy (82, 83). Interestingly, this has also been confirmed for pelvic osteosarcoma, an anatomic location which is associated with bad prognosis *per se*. The main purpose of chemotherapy is to reduce the micrometastatic tumour burden. However, there are patients whose tumour cells are completely unresponsive to these drugs. The reasons for this are currently unknown, however, it has been shown that there is an inbuilt cellular expression profile which determines the fate regarding the chemotherapy response already in the primary tumour (97). Although currently still too expensive to be used on a daily basis, there is great potential in the future to distinguish those patients who will benefit from chemotherapy from others, thereby sparing them from the hazardous treatment. Basically, besides the development of metastasis and unresponsiveness to chemotherapy as the two most important prognostic indicators, all other prognostic parameters described for dogs are also applicable to humans and are only mentioned in the following part of this review.

A number of factors have been identified as prognostic in dogs with osteosarcoma. The most important ones include presence of metastases at the time of diagnosis (11), alkaline phosphatase (ALP) levels (98, 99), histological grade (100) and microvascular density (101). Once metastatic disease is clinically detectable, chemotherapy is usually ineffective at improving survival (102). Pulmonary metastasectomy is a well-described procedure in people (103) that was shown to contribute to significant prolonged survival in a small subset of canine patients (104).

ALP-levels are a negative prognostic factor and dogs with elevated levels have shorter survival times by approximately 50% (170 days *versus* >400 days), even when treated aggressively with surgery and chemotherapy (98, 99).

There are many different histological subclassifications of osteosarcoma based on the type and amount of matrix and the characteristics of the cells. So far, it has not been well established if these subclassifications have any prognostic significance (13, 105). It has been shown that there is a correlation between the metastatic behaviour of canine osteosarcoma and the histological grade, based on microscopic features like cellular pleomorphism, mitotic figures, tumour matrix, tumour cellularity and percentage necrosis (100, 106). Twenty-five percent of tumours that were categorized as grade I or II were associated with a significantly longer disease-free and overall survival than the 75% of tumours graded as III. One study of 52 canine osteosarcoma samples revealed that dogs presenting with detectable metastatic disease had a significantly higher vascular density in their primary tumour than those presenting without (101).

Young dogs appear to have shorter survival times, with biologically more aggressive disease (91). The reason for a poorer prognosis in very young dogs is unknown, but it has also been documented in humans (107-111). It should be noted that although osteosarcoma in people usually affects young adults and skeletally immature children and adolescents, in dogs, osteosarcoma almost always affects skeletally mature individuals. Therefore, although the youngest affected individuals of both species seem to have the poorest prognosis, the biological basis for this effect may be different in each species.

Tumour necrosis after primary chemotherapy is a well recognized prognostic factor in human medicine. Percentage necrosis is used to modify postsurgical chemotherapy protocols. In one study with 200 dogs, tumour necrosis was strongly correlated with local tumour control, but there was no correlation between tumour necrosis and time to metastasis (112). Another study found a significant direct correlation between survival time and percentage necrosis following 2 or 3 doses of doxorubicin (88).

Other factors have been proposed as prognostic indicators for canine osteosarcoma. Large tumour size (13, 113, 114) has been correlated with poor outcome in dogs and has been reported as a negative prognostic factor in human osteosarcoma (115). In dogs, body weight >40 kg (17, 105) and proximal humerus location were associated with a shorter disease-free interval and survival in another study (116). Possible explanations for these findings are that tumours in the proximal humerus might be more advanced before detection and that larger dogs might receive proportionally less chemotherapy than smaller dogs, when treated on a per meter-square body surface area basis. In contrast, the only anatomic site that was associated with an improved outcome was the mandible (117).

Molecular prognostic factors. Although *p53* alterations alone were not shown to be a useful marker for disease progression in humans (10), in one study, the coexpression of *p53* and p-glycoprotein was found to be a strong negative prognostic factor (118). In one study, 167 canine osseous tumours were evaluated for clinicopathological value of immunohistochemical expression of *p53* protein (119). The authors found that the prevalence of overall positive *p53* staining was higher in osteosarcoma than other bone tumours. There was a direct correlation between *p53* index (= *p53* staining frequency and intensity) and clinicopathological parameters, for example the histologic grade of osteosarcomas, the mitotic index and degree of tumour necrosis. *P53* index showed no association with age, primary or secondary site status, or the presence of metastases. The authors considered the *p53* index as a potential prognostic indicator for canine osseous tumours.

In 47 human osteosarcoma patients, *Rb* gene loss of heterozygosity was correlated with a significantly lower event-free survival rate (49). In another study, *Rb* gene abnormalities were associated with tumour grade (120). *Rb* gene loss of heterozygosity was considered an early predictive feature for patients with osteosarcoma indicating a potentially unfavourable prognosis.

COX-2 is an inducible enzyme, responsible for the production of prostaglandin E_2 (PGE_2), which has been found to be up-regulated in inflammatory and neoplastic conditions (121, 122). A study in human pediatric sarcomas including osteosarcomas found that 86% of tumours overexpressed COX-2 and there was a trend towards increased COX-2 expression in metasatic osteosarcoma (123) However, Cox-2 expression did not vary with clinicopathological features and was not predictive of prognosis in another study of 99 archived human osteosarcoma samples (124). A recent *in vitro* study even found decreased proliferation and increased apoptosis in human osteosarcoma cells overexpressing COX-2 (125). COX-2 expression was analysed in 44 canine osteosarcoma (126) and 77.3% of the tissue samples were positive for COX-2 expression, although most of the cases (88%) had poor to moderate COX-2 staining. Dogs with strong COX-2 expression had significantly decreased overall survival compared to dogs with negative or poor to moderate staining (86 days *vs.* ~400 days).

Treatment Options and Outcome

Standard of care. Standard of care, defined as the treatment that results in the longest median survival times, is a combination of chemotherapy and surgery. In humans, neoadjuvant chemotherapy is followed by surgical resection of the tumour and adjuvant chemotherapy. This treatment strategy leads to a 5-year survival of 60% overall. Seventy-five percent of patients with localized disease are alive after 5 years, whereas the 5-year survival rate for patients with metastatic disease at presentation is only 15 to 20%. In dogs, surgical resection of the primary tumour, followed by 3 to 6 cycles of either a platinum- or doxorubicin-based chemotherapy protocol is considered the standard therapy (88, 89, 116, 127-130). This treatment results in a 50% 1-year survival and a 20% 2-year-survival (11).

Palliative treatment. Due to the established treatment regimen in humans with osteosarcoma, palliative treatment is rarely indicated nowadays. Widespread metastatic disease responds poorly to chemotherapy. Locally uncontrolled tumour growth may lead to chronic pain und poor function of the respective limb, and amputation may be indicated under these circumstances.

Palliation is indicated for dogs with metastatic osteosarcoma or when owners do not want to pursue more aggressive treatment options. Palliative management aims

to control pain and lameness associated with the primary tumour but does not attempt to modify disease progression or improve survival time.

Radiation therapy provides effective symptomatic treatment for local bone pain in humans and dogs. It reduces inflammation and results in tumour cell necrosis, replacement by fibrous tissue, and formation and calcification of woven bone (131). Radiation may be used in humans when there is a surgically inaccessible or uncontrollable lesion such as the pelvis or spine. A number of different radiation protocols were described (132-135). The overall response rates range from 74% to 92% with median duration of response intervals ranging from 73 days to 130 days and median survival ranging from 122 to 313 days.

Analgesia is the cornerstone of palliative management of dogs with osteosarcoma. Initially, NSAIDs may be sufficient to control pain and improve quality of life (136). More potent analgesics (*e.g.* opiates) and combinations of these drugs are often required for effective pain relief during the course of therapy (136). Analgesics can also be used together with radiotherapy.

Future therapeutic strategies. Despite significant improvements over the last decades using a combination of chemotherapy and surgery, patients with metastatic disease and/or unresponsiveness to chemotherapy continue to have a very poor prognosis. Understanding the mechanisms and interactions of various molecular pathways in patients with osteosarcoma will hopefully help to identify new targets for therapy that may prove more effective or less toxic , especially for the tumours that respond poorly to conventional treatment (137, 138). New treatment strategies under investigation are, for example, molecular targeting of metastasis or chemoresistance markers, investigations in nanomedicine, and immunostimulatory agents that might be of benefit in the treatment of osteosarcoma.

Results from studies in canine patients have provided relevant information for new treatment strategies in human osteosarcoma. A randomized, double-blind study performed in canine spontaneous osteosarcoma showed that muramyl tripeptide (MTP), a macrophage activator, significantly prolonged survival in dogs after amputation (139). Subsequent clinical studies in human patients with metastatic osteosacoma demonstrated a longer time to relapse following metastatectomy after administration of liposomal MTP (140-142).

The safety and toxicity of intravenous gene delivery, using liposome-DNA complexes encoding the canine *IL-2* gene was evaluated in a phase-I study in 20 dogs with metastatic osteosarcoma. The IV-infusions were well tolerated, capable of eliciting substantial immune activation and able to prolong survival times in dogs with advanced lung metastases (143).

Conclusion

Osteosarcoma in dogs shows striking similarities in tumour biology and behaviour, including metastatic prospensity, with its human counterpart. Therefore, canine patients offer a unique opportunity for autochthonous tumour studies. Further understanding of important similarities and differences from the human disease are necessary to define limitations and advantages of using domestic dogs as models for human osteosarcoma.

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