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Review

Prognostic and predictive biomarkers of canine osteosarcoma

Gayathri Thevi Selvarajah [a,b], Jolle Kirpensteijn [a,*]

[a] Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, 3584CM Utrecht, The Netherlands
[b] Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine, University Putra Malaysia, 43400 Serdang, Malaysia

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ABSTRACT

Canine osteosarcoma (OS) is an aggressive tumour that accounts for approximately 90% of primary bone tumours in the dog. Although the standard treatments (including limb amputation/sparing, chemotherapy and palliative radiotherapy) have significantly increased survival rates, almost 90% of animals will eventually develop predominantly pulmonary metastases. Despite advances in various therapies, prognosis remains poor, with median survival times ranging from 3 months to 1 year and <20% of dogs survive for >2 years following diagnosis.

Various clinical and epidemiological markers have facilitated decision-making with respect to therapy but no single molecular biomarker has been shown to enhance prediction of disease progression. The publication of the canine genome in 2005 raised the possibility of increasing understanding of the genetic mechanisms underpinning canine OS. This review explores the use of biomarkers within the multi-disciplinary management of dogs with OS, and highlights the few known, potential prognostic/predictive molecular markers including their potential value as 'bridging biomarkers' for human OS. Although high-throughput profiling of canine OS remains in its infancy, research within the next decade using leading-edge screening technologies has the potential to identify biomarkers that may enhance diagnostic and prognostic accuracy and result in more effective, individually tailored, treatment and management protocols for affected dogs.

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Introduction

Osteosarcoma (OS) is a malignant neoplasm of mesenchymal origin that produces osteoid and accounts for approximately 85% of all primary canine bone tumours. A heterogeneous tumour with regard to location, metastatic sites, radiological presentation, histopathological subtypes, progression and response to treatment, it is almost exclusively observed in large or giant breeds such as the Rottweiler, Great Dane, Greyhound, Saint Bernard and Doberman pinscher (Norrdin et al., 1989; Ru et al., 1998; McNeill et al., 2007). There is some evidence to suggest males are more predisposed to OS and the median age of onset of clinical signs ranges from 8 to 10 years (Spodnick et al., 1992; Boston et al., 2006), although a subset of tumours arises in younger dogs (Evans, 1983).

Dogs often present with a history of lameness or even fracture of the affected bone. Predilection sites are the weight-bearing regions of the long bones (humerus, femur, radius, tibia and ulna) (Liptak et al., 2004) with approximately 25% of tumours arising in the axial skeleton including the flat bones of the skull, ribs, vertebrae, sternum, and pelvis (Hammer et al., 1995; Dickerson et al., 2001). Intriguingly, given that OS is a 'sarcoma of bone', primary tumours arising at extra-skeletal sites have also been described (Kuntz et al., 1998; Langenbach et al., 1998).

OS is an aggressive and invasive neoplasm that causes local skeletal destruction resulting in radiographic evidence of both osteoproductive and osteolytic lesions. It is highly metastatic, predominantly to the lungs with a lower frequency of spread to distant bones, regional lymph nodes (Hillers et al., 2005) and other soft tissues (Peremans et al., 2003; Gorman et al., 2006). A clinical diagnosis is made following assessment of case signalment and history and based on the radiographic appearance of the lesion.

The current diagnostic 'gold standard' for OS is histopathological examination with tumour classification based on the formation of osteoid matrix with osteoblastic, fibroblastic, chondroblastic and telangiectic subtypes (Kirpensteijn et al., 2002; Loukopoulos and Robinson, 2007). There can be considerable variation in the histological appearance both between and within individual neoplasms. Metastatic lesions usually appear histologically identical to the primary tumour, although they more frequently exhibit a greater degree of necrosis. The histopathological grading system employed by Kirpensteijn et al. (2002) demonstrated that grade III tumours, which account for 75% of cases, have a significantly poorer prognosis than grade I and II neoplasms. However, this grading system was not significantly prognostic for tumours from non-appendicular sites.

* Corresponding author. Tel.: +31 302531776; fax: +31 302518126.
E-mail address: J.Kirpensteijn@uu.nl (J. Kirpensteijn).

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Historically, dogs with OS that were treated by amputation alone had poor overall survival times (ST), typically <3 months, with the majority dying or being euthanased due to metastatic disease (Brodey and Abt, 1976). Over the years, although there have been advances in disease management, amputation remains the basic standard-of-care for appendicular OS with 'limb-sparing' used only to selectively remove tumours located in the distal radius, ulna and tibia (Straw and Withrow, 1996; Boston et al., 2007). With primary tumour removal, dogs will be pain-free for a short time, but 90% succumb to metastatic disease within 3–6 months.

Adjuvant therapy, such as multi-modal chemotherapy regimes, treatment with bisphosphonates or immune modulators and palliative radiation, is now provided at specialised veterinary practices (Tomlin et al., 2000; Dow et al., 2005; Walter et al., 2005; Fan et al., 2009). Combined therapy can contribute significantly to ST, with 40–50% of dogs surviving >1 year when treated by amputation in combination with chemotherapy (Bergman et al., 1996; Phillips et al., 2009), although <10–20% survive >2 years. Finally, although not widely practiced due to operative and post-operative complications, a subset of dogs with less than three pulmonary metastatic lesions are reported to benefit from metastasectomy (O'Brien et al., 1993).

Over the years, various compounds have been used in adjuvant chemotherapy including cisplatin, carboplatin and doxorubicin. These have been used in single and in multi-agent regimes and at varying dosage and treatment interval (Berg et al., 1995; Bergman et al., 1996; Chun et al., 2000; Bailey et al., 2003). No differences in survival are seen when these treatments are compared with pre- or post-operative chemotherapy (Berg et al., 1997) and no differences in the disease-free interval (DFI) have been reported for dogs treated using single- or multi-agent chemotherapeutic regimes. Prolonged, intense use of chemotherapy is often not an option due to adverse side-effects compromising any clinical benefits (Barabas et al., 2008), and to date, aggressive therapy has proven ineffective in restricting the growth of metastases. A small number of cases of canine OS that do not receive adjuvant chemotherapy, do not succumb to metastatic disease once the primary tumour has been removed (Selvarajah et al., 2009). This suggests that genetic composition of both the host and tumour may contribute to differences in the metastatic potential. In this regard it is important to differentiate patients that respond to chemotherapy, from those that do not.

There are many documented prognostic indicators for canine OS (Table 1), and the majority of these are similar to those reported from large retrospective studies of the human neoplasm, including age, tumour size, location and histological subtypes (Owen, 1967; Kim et al., 2007; Bramer et al., 2009; Pakos et al., 2009). The accurate segregation of canine patients into distinct prognostic subgroups, based on such indicators, is key to the tailoring of appropriate treatment.

The potential use of biomarkers in the management of canine OS

Biomarkers are central to 'personalised' medicine. One definition of a biomarker is that it is a specific 'measure' of a biological/pathological process or cellular response to a particular therapy or stage of the disease. Advances in our understanding of the canine genome have provided opportunities to enhance our knowledge of the molecular basis of pathogenesis and progression of dog cancers. Gene expression data allows us to further classify animals with OS and to use statistical analysis to enhance clinical decision-making. The evaluation of such patients based on clinical need rather than on other aspects of the disease state is vital. Many proteins that could reflect disease are not released or do not leak from diseased tissue into the circulation, and biomarkers are typically those substances that can be detected in samples such as serum, plasma or urine.

Over the last decade, increased molecular-based research has improved our understanding of the pathogenesis of both canine and human OS. Many investigations have been directed at identifying single gene alterations as predictors of metastasis, cell proliferation, drug resistance, bone turnover and other processes central to disease progression. In this context we propose a conceptual framework for the potential use of such biomarkers as diagnostic and prognostic indicators as well as their use in therapeutic decision-making in the management of dogs with OS (Fig. 1).

It is likely that reliable biomarkers will be those useful in multiple species, so-called translational or 'bridging' biomarkers. Indeed, canine OS provides an excellent model for studying this neoplasm in humans (Paoloni et al., 2009) since the tumour has features common to both species including clinical presentation, histopathological appearance, location and sites of metastases and prognosis. However, few compounds to date qualify as such 'bridging' biomarkers, largely because of the paucity of research on the canine form. Furthermore, while many biomarkers have potential translational applications, most of these have been based on functional, *in vitro* assays and have not been validated by retrospective or prospective studies.

Following a search of peer-reviewed publications from 1954 to 2009 using the National Center for Biotechnology Information (NCBI) Entrez Pubmed search engine[1] and the Web of Knowledge[2], and using the keywords 'CANINE', 'DOG', 'OSTEOSARCOMA' and 'OSTEOGENIC SARCOMA', retrieved articles were assessed for 'molecular' prognostic indicators/predictive markers for OS. The predictive and translational relevance/significance of the identified potential biomarkers is discussed below.

Diagnostic biomarkers for canine osteosarcoma

The differential diagnosis of OS has become increasingly reliant on molecular diagnostics and immunohistochemistry, although currently there are no biomarkers that can reliably classify the histopathological subtype or predict the malignant potential of canine bone tumours. Alkaline phosphatase is an unreliable diagnostic biomarker for OS as it cannot differentiate this tumour from other bone-forming tumours such as multilobular osteochondrosarcoma or from reactive bone lesions (Barger et al., 2005). Ezrin, a cytoskeletal linker protein, has recently been reported to have 100% specificity in differentiating human chondroblastic OS from chondrosarcoma. This form of OS expressed this protein regardless of histology grade (Salas et al., 2009) and ezrin is an example of how molecular-based markers can facilitate diagnostic precision. The use of this biomarker in differentiating the histological variants in canine OS remains to be determined.

Prognostic and predictive biomarkers

Prognostic biomarkers help predict patient survival and overall clinical outcome. In consequence, these markers should reflect particular biological properties of the neoplasm such as their proliferative and metastatic capacities, and should also be able to segregate tumours/dogs into various subgroups based on likely disease-course. Predictive biomarkers are those that aid clinicians in assessing if a dog will respond favourably to a particular therapy (Gogas et al., 2009). The capacity to stratify dogs into prognostic

[1] See: www.ncbi.nlm.nih.gov/.
[2] See: www.isiknowledge.com/.

Table 1
A summary of clinical and histopathological predictors or prognosticators of outcome for dogs with osteosarcoma (OS).

Study (reference)	Prognostic factor	Summary/interpretation(s)
Boston et al. (2006)	Lung metastasis	Grave prognosis, euthanasia offered
Hillers et al. (2005)	Lymph node metastasis	Rare in dogs but those with lymph node metastasis have significantly poorer prognosis in terms of DFI[a] and ST[b]
Kirpensteijn et al. (2002) Hammer et al. (1995) Moore et al. (2007)	Tumour mitotic index	Increased mitotic index reduces DFI
Lascelles et al. (2005)	Post-operative infection	Post-operative infection after limb-sparing surgery can increase ST
Misdorp and Hart (1979) Forrest et al. (1992)	Increased tumour size	Increasing tumour size significantly associated with pulmonary metastasis and poor prognosis
Misdorp and Hart (1979)	Extension of tumour into adjacent soft tissue	Poor prognosis
Berg et al. (1995) Powers et al. (1991) Misdorp and Hart (1979) Loukopoulos and Robinson (2007)	Percentage of tumour that is necrotic Histological subtype	Significant direct correlation with ST. Percentage of tumour that is necrotic strongly predictive of local tumour control but no correlation with time for metastasis Fibrosarcomatous subtype has a more favourable prognosis
Loukopoulos and Robinson (2007) Moore et al. (2007) Spodnick et al. (1992)	Age	Dogs $\leqslant$ 5 years old have shorter DFI compared to older dogs. The mitotic index is higher in tumours from young dogs
Bergman et al. (1996)	Tumour location: humerus	Dogs with tumours involving the humerus have shorter DFI and ST
Kirpensteijn et al. (2002)	Histological grade	Higher grades associated with decreased ST and DFI. Grade I and II tumours have a significantly better prognosis relative to grade III
Kirpensteijn et al. (2002) Selvarajah et al. (2009) Hillers et al. (2005) Garzotto et al. (2000) Vail et al. (2002) Ehrhart et al. (1998) Moore et al. (2007)	Serum alkaline phosphatase	Increased plasma levels of this enzyme associated with shorter DFI and ST
Dickerson et al. (2001) Straw et al. (1996)	Tumour location: mandible	More favourable prognosis. Dogs treated with surgery alone had 1 year survival rate of 71%, which is higher than for dogs with appendicular OS
Bergman et al. (1996) Lascelles et al. (2005) Moore et al. (2007)	Bodyweight	Dogs of lower body weight (<40 kg) had significantly longer DFI and ST
Kirpensteijn et al. (2002)	Vascular invasion	Reduced DFI

[a] DFI, disease-free interval.
[b] ST, survival time.

and predictive subgroups thus greatly facilitates the delivery of optimal therapy. Although still at the developmental stage, gene expression profiles have been used in such patient stratification (Selvarajah et al., 2009).

Elevated total alkaline (TALP) and bone alkaline phosphatase (BALP) concentrations are known prognostic indicators for canine OS. Dogs with normal pre-treatment TALP and BALP levels survived significantly longer than did animals with increased pre-treatment levels of this enzyme (Garzotto et al., 2000). Although the pre-operative elevation of alkaline phosphatase can be used as a prognostic biomarker in dogs, there are discrepancies as to its use post-operatively in predicting metastases or tumour recurrence.

To date, the molecular markers that have been investigated for canine OS reflect the various genetic alterations of the tumour with respect to disease progression. Few appear prognostically useful because of lack of independent validation and significant further investigation is required in this area.

TP53 tumour suppressor

Tumour suppressor genes encode proteins that prevent or retard cell division, and their mutation contributes to the development and progression of cancer. Commonly mutated tumour suppressors found in canine OS include phosphatase and tensin homolog (PTEN) and tumour protein 53 (TP53). Although PTEN mutations and subsequent down-regulation of protein expression are present in a majority of canine cell lines and tumours, no prognostic significance to this event has been reported (Levine et al., 2002). However, TP53 mutational inactivation has been described in both *in vitro* models and in spontaneous OS in the dog (van Leeuwen et al., 1997; Johnson et al., 1998).

Elevated levels of TP53 protein are commonly encountered in tissues harbouring cells containing the mutation. In 84% of appendicular and 56% of axial cases of canine OS, there is elevated TP53 expression (Sagartz et al., 1996; Loukopoulos et al., 2003). In one independent study population, TP53 had mutated in approximately 40% of tumours from both axial and appendicular sites; this mutation status had prognostic value as dogs with mutated TP53 had significantly shorter ST that correlated with elevated serum alkaline phosphatase concentration and tumour histological grade (Kirpensteijn et al., 2008). To date, it remains unclear if the TP53 mutation occurs in the metastases as well as in the primary tumour (van Leeuwen et al., 1997; Kirpensteijn et al., 2008), although it is quite possible that other events, rather than this mutation, contribute to tumour spread (Vousden, 2002). The TP53 mutation may be an independent prognostic indicator and predictor of a more malignant phenotype of neoplasm which could be used in prospective patient evaluation.

Angiogenic markers: vascular endothelial growth factor

Plasma vascular endothelial growth factor (VEGF) is associated with more aggressive tumours in dogs (Wergin and Kaser-Hotz, 2004) and for OS, VEGF concentrations significantly correlate with

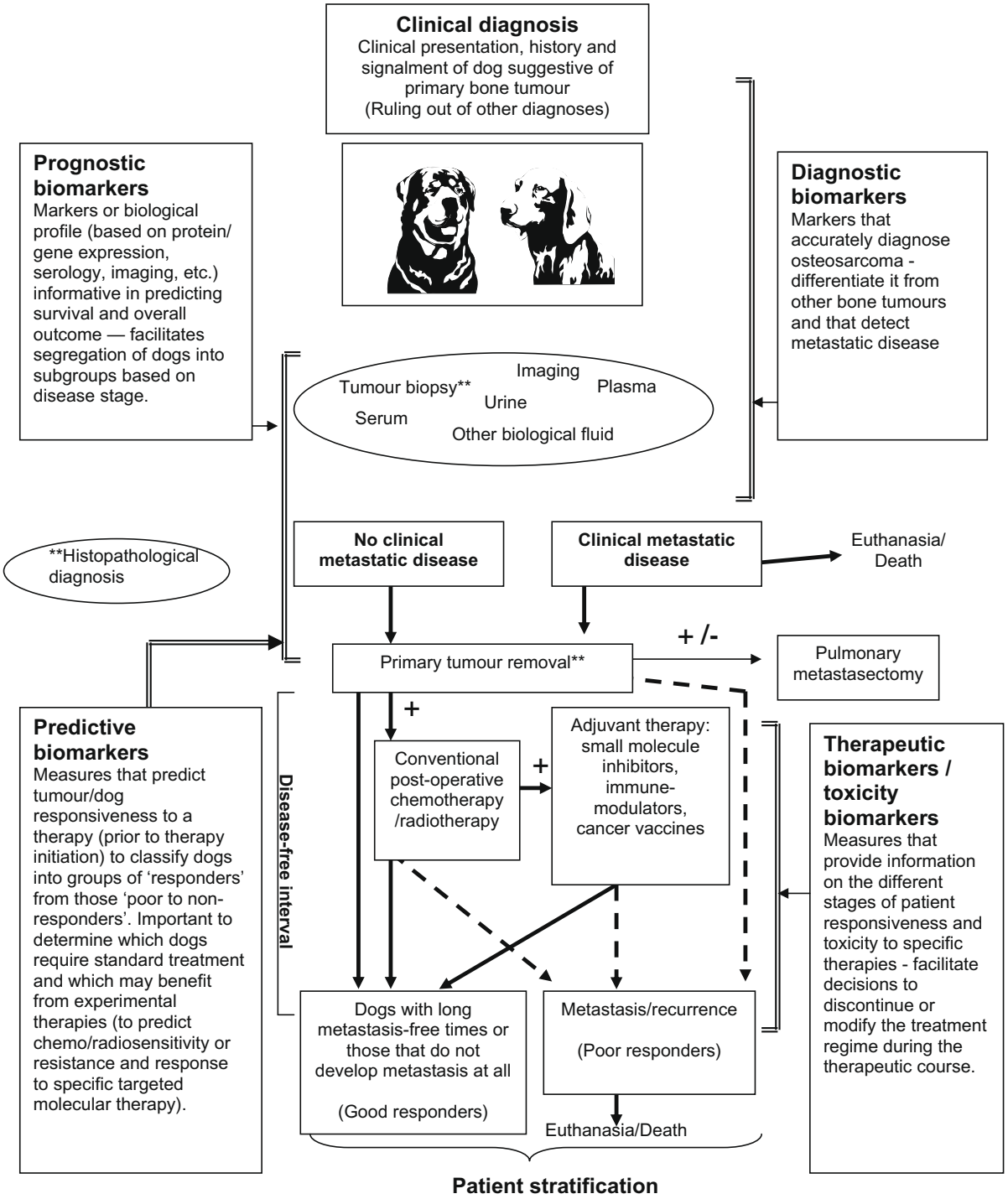

Fig. 1. The potential use of various biomarkers within the multi-modal management of dogs with osteosarcoma.

DFI (Thamm et al., 2008). Similarly, VEGF expression in resected human OS is an important negative prognostic factor (Bajpai et al., 2009), and strong predictor of metastasis and poor survival in the 'chemo-naïve' human neoplasm (Kaya et al., 2000). The VEGF pathway has a number of critical members, particularly receptors that play significant roles in angiogenesis and hence characterise the malignant tumour phenotype. VEGF receptor (R)-3 negatively correlates with ST and DFI in human OS (Abdeen et al., 2009), and VEGFR-2 links with candidate genes associated with poor prognosis in canine OS on pathway analysis (Selvarajah et al., 2009).

Mesenchymal–epithelial transition factor

Mesenchymal–epithelial transition factor (MET) is a proto-oncogene that encodes a protein also known as c-Met or hepatocyte growth factor receptor (HGFR). MET has many roles in neoplasia including an ability to activate oncogenic pathways and to participate in angiogenesis and metastasis. MET is expressed in canine OS including lung metastases (Ferracini et al., 2000), and expression of MET in primary tumours was found to predict for metastasis via lymphatics (Fieten et al., 2009). MET mRNA is constitutively expressed by both human and canine OS cell lines (De

Maria et al., 2009), where stimulation with hepatocyte growth factor results in increased cellular proliferation and the formation of larger *in vitro* cell colonies (MacEwen et al., 2003). Small molecule inhibitors of MET have been shown *in vitro* to impair the invasive properties of canine OS cells and may represent a future treatment option (De Maria et al., 2009).

Cyclooxygenase-2

Cyclooxygenase (COX)-2 is an enzyme involved in apoptosis, immune surveillance and in angiogenesis and has been implicated in the production of prostaglandins in tumours including OS (Mohammed et al., 2004). The value of COX-2 as a biomarker in human OS is debatable as a predictor of metastasis and ST (Dickens et al., 2003; Rodriguez et al., 2008; Urakawa et al., 2009). In cases of canine appendicular OS, dogs with tumours with strong expression of COX-2 had a significantly decreased overall ST (86 days) relative to dogs with tumours with minimal expression of this enzyme (>300 days) (Mullins et al., 2004). However, since the number of dogs investigated in this study was small, more extensive studies will be required to validate this finding.

Ezrin

As outlined above, ezrin is a cytoskeletal linker protein, a member of the ezrin–radixin–moesin (ERM) protein family, which has a key role in the coordination of tumour metastasis (Hunter, 2004). An immunohistochemical study on canine OS revealed that high expression of this protein is associated with early metastases, and hence poorer clinical outcome. Consistent with this finding, a significant association between high ezrin expression and poor outcome is reported in paediatric OS (Khanna et al., 2004). In other studies of primary human OS, ezrin over-expression predicted lung metastasis (Xu-Dong et al., 2009) and, when associated with elevated alkaline phoshatase levels, predicted patients that responded to chemotherapy but had poor overall survival relative to patients with tumours that did not express this protein (Kim et al., 2009a).

Metalloproteinases

Metalloproteinases (MMP) are zinc-dependent enzymes commonly expressed in neoplasia and in inflammatory disease. In the context of cancer, MMP have been implicated as biomarkers of shorter DFI (Uchibori et al., 2006), and as predictors of survival following neo-adjuvant chemotherapy (Foukas et al., 2002). These enzymes also correlate with the invasive and metastatic forms of human OS, and in particular, MMP-2 and -9 have been shown to be highly expressed in three canine OS cell lines (Loukopoulos et al., 2004). Primary OS in 30 dogs had greater MMP expression in tumour than in stromal cells, suggesting a role for the enzyme in malignancy (Lana et al., 2000). Although an elevated plasma MMP-2 concentration has been linked with poor prognosis, its inhibition with BAY 12-9566, in combination with doxorubicin chemotherapy, did not improve ST in dogs with OS (Moore et al., 2007).

Chemoresistance markers

ST for dogs with OS has remained static for the last decade despite advances in chemotherapy and has been attributed in part to the chemoresistance of the neoplasm. In treating human OS, two well-characterised molecular markers of multi-drug resistance are used: the drug resistance pump P-glycoprotein (P-gp), and the multi-drug resistance-related protein 1 (MRP1). P-gp, a substrate for MRP1, is expressed in canine OS cell lines resistant to doxorubicin (Page et al., 2000) and in 66% of cases of canine OS (Cagliero et al., 2004), but its prognostic or predictive value of ST has not been determined. Recently, gene expression profiling of canine OS revealed up-regulation of transcripts associated with drug resistance such as MGST1 (Selvarajah et al., 2009). Future elucidation of the chemoresistance mechanisms operational in canine OS will be key to circumventing this problem.

Therapeutic and toxicity biomarkers

Therapeutic and toxicity biomarkers monitor the ongoing effects of a compound on a patient. Haematology, serology, urine biochemistry, blood gas analysis and histopathology are currently used to monitor patients for adverse effects on cardiac, renal and hepatic function (Barabas et al., 2008). Molecular approaches may be able to precisely determine the effect of particular therapies during OS progression. These could include markers of bone remodelling, such as the collagen breakdown products N- and C-telopeptide, which could monitor dogs undergoing anti-resorptive therapies (Lacoste et al., 2006; Fan et al., 2007; Lucas et al., 2008). Similarly, serum concentrations of cardiac troponin I (cTnI), a sensitive and specific marker of cardiomyocyte death, could be used to monitor dogs on post-operative chemotherapy, especially animals receiving the known cardiotoxin doxorubicin (Selting et al., 2004). Studies to discover novel therapeutic and toxicity biomarkers can be performed in parallel with clinical trials where the serial assessment of various molecular markers is being carried out.

Integrative platforms for biomarker discovery: pitfalls and prospects

State-of-the-art technologies will be required to carry out non-invasive, *in vivo* serial assessment of disease progression, therapeutic response and drug toxicity in dogs with OS. The advent of high-throughput 'genomic' assessment has raised questions as to whether single or multiple markers are most appropriate. In most circumstances, given tumour heterogeneity and the variation in stage of development, single biomarkers are unlikely to be sufficiently sensitive and specific and it is likely that panels of biomarkers will be required.

We have recently published the novel correlation of gene expression profiling with prognosis for canine OS (Selvarajah et al., 2009). The differences in gene expression profile between two survival groups were minor (<100 genes) compared to similar studies of human OS, where large numbers of candidate genes were identified (Nakano et al., 2003; Srivastava et al., 2006; Walters et al., 2008). The genes that were found to have prognostic significance in the canine study had roles in cell proliferation, drug resistance and in metastasis, which, taken together, reflect tumour malignancy. The study also identified subgroups of animals that correlate highly with ST. Although the sample size was small, these findings provide initial insights that merit further investigation and cross-validation with studies in humans.

The immunohistochemical labelling of particular proteins in tumours provide information regarding tumour nature and behaviour. However, limitations in the numbers of specific, labelling antibodies that can be used in dogs restrict the usefulness of this methodology. Tissue arrays consisting of panels of >100 'outcome-linked' tumour tissue punctures on a single slide can be used to screen for novel biomarkers. Such an approach can be used to screen large numbers of patients and can determine if particular proteins are co-expressed. Multiple 'spots' within an OS tumour have to be assessed, because of the heterogeneous nature of this neoplasm and because expression of a protein marker in a single spot may not represent its expression in the tumour as a whole. Although prognostic biomarker discovery using immunohisto-

chemistry is increasingly popular, standardised methods across veterinary laboratories including antigen retrieval methods, types of antibodies used (polyclonal or monoclonal), incubation periods and staining evaluation criteria have not been established.

Despite advances in diagnostic imaging, micrometastases present at the onset of disease are frequently not detected. Methods need to be developed to detect neoplastic cells in the circulation and thus predict metastatic disease based on the primary tumour protein expression 'signature'. In this context, *in vitro* metastatic sub-clone models are useful in elucidating key transcripts and signalling pathways important in the survival of metastatic cells. The gene profiling of such models of canine OS are underway and it is anticipated these will identify pathogenic mechanisms and targets for therapy. Although *in vitro* models are frequently used in the discovery of biomarkers for OS, different cell lines may contain different genetic alterations and activated signalling pathways, raising questions as to how closely these mimic the *in vivo* situation.

Comparative genomic hybridisation cytogenetic array analysis (Thomas et al., 2005), cDNA/oligonucleotide microarray gene expression profiling, protein profiling using mass spectrometry, and miRNA profiling are all currently used to investigate canine OS and in the future, cancer lipidomic (Wenk, 2005; Fernandis and Wenk, 2009) and metabolomic (Kim et al., 2009b) profiling approaches could be used in the pursuit of novel biomarkers.

Conclusions

The study of spontaneously occurring tumours of the dog, such as OS, provides invaluable translational opportunities for human medicine, particularly in the potential discovery of novel, 'bridging biomarkers'. The few molecular biomarkers with predictive and prognostic value so far identified in dogs require validation on a larger scale which can be achieved given appropriate collaboration between the pharmaceutical industry, biomedical and veterinary scientists within academia, and veterinarians working in clinical settings. An exciting decade of biomarker discovery using high-throughput methodologies is now anticipated, and it is hoped that this will result in more effective, individually tailored, treatment and management protocols for affected dogs.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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References

Abdeen, A., Chou, A.J., Healey, J.H., Khanna, C., Osborne, T.S., Hewitt, S.M., Kim, M., Wang, D., Moody, K., Gorlick, R., 2009. Correlation between clinical outcome and growth factor pathway expression in osteogenic sarcoma. Cancer 115, 5243–5250.

Bailey, D., Erb, H., Williams, L., Ruslander, D., Hauck, M., 2003. Carboplatin and doxorubicin combination chemotherapy for the treatment of appendicular osteosarcoma in the dog. Journal of Veterinary Internal Medicine 17, 199–205.

Bajpai, J., Sharma, M., Sreenivas, V., Kumar, R., Gamnagatti, S., Khan, S.A., Rastogi, S., Malhotra, A., Bakhshi, S., 2009. VEGF expression as a prognostic marker in osteosarcoma. Pediatric Blood and Cancer 53, 1035–1039.

Barabas, K., Milner, R., Lurie, D., Adin, C., 2008. Cisplatin: a review of toxicities and therapeutic applications. Veterinary and Comparative Oncology 6, 1–18.

Barger, A., Graca, R., Bailey, K., Messick, J., de Lorimier, L.P., Fan, T., Hoffmann, W., 2005. Use of alkaline phosphatase staining to differentiate canine osteosarcoma from other vimentin-positive tumors. Veterinary Pathology 42, 161–165.

Berg, J., Weinstein, M.J., Springfield, D.S., Rand, W.M., 1995. Results of surgery and doxorubicin chemotherapy in dogs with osteosarcoma. Journal of American Veterinary Medical Association 206, 1555–1560.

Berg, J., Gebhardt, M.C., Rand, W.M., 1997. Effect of timing of postoperative chemotherapy on survival of dogs with osteosarcoma. Cancer 79, 1343–1350.

Bergman, P.J., MacEwen, E.G., Kurzman, I.D., Henry, C.J., Hammer, A.S., Knapp, D.W., Hale, A., Kruth, S.A., Klein, M.K., Klausner, J., Norris, A.M., McCaw, D., Straw, R.C., Withrow, S.J., 1996. Amputation and carboplatin for treatment of dogs with osteosarcoma: 48 cases (1991 to 1993). Journal of Veterinary Internal Medicine 10, 76–81.

Boston, S.E., Ehrhart, N.P., Dernell, W.S., Lafferty, M., Withrow, S.J., 2006. Evaluation of survival time in dogs with stage III osteosarcoma that undergo treatment: 90 cases (1985–2004). Journal of the American Veterinary Medical Association 228, 1905–1908.

Boston, S.E., Duerr, F., Bacon, N., Larue, S., Ehrhart, E.J., Withrow, S., 2007. Intraoperative radiation for limb sparing of the distal aspect of the radius without transcarpal plating in five dogs. Veterinary Surgery 36, 314–323.

Bramer, J.A., van Linge, J.H., Grimer, R.J., Scholten, R.J., 2009. Prognostic factors in localized extremity osteosarcoma: a systematic review. European Journal of Surgical Oncology 35, 1030–1036.

Brodey, R.S., Abt, D.A., 1976. Results of surgical treatment in 65 dogs with osteosarcoma. Journal of the American Veterinary Medical Association 168, 1032–1035.

Cagliero, E., Ferracini, R., Morello, E., Scotlandi, K., Manara, M.C., Buracco, P., Comandone, A., Baroetto Parisi, R., Baldini, N., 2004. Reversal of multidrug-resistance using Valspodar (PSC 833) and doxorubicin in osteosarcoma. Oncology Reports 12, 1023–1031.

Chun, R., Kurzman, I.D., Couto, C.G., Klausner, J., Henry, C., MacEwen, E.G., 2000. Cisplatin and doxorubicin combination chemotherapy for the treatment of canine osteosarcoma: a pilot study. Journal of Veterinary Internal Medicine 14, 495–498.

De Maria, R., Miretti, S., Iussich, S., Olivero, M., Morello, E., Bertotti, A., Christensen, J.G., Biolatti, B., Levine, R.A., Buracco, P., Di Renzo, M.F., 2009. Met oncogene activation qualifies spontaneous canine osteosarcoma as a suitable pre-clinical model of human osteosarcoma. The Journal of Pathology 218, 399–408.

Dickens, D.S., Kozielski, R., Leavey, P.J., Timmons, C., Cripe, T.P., 2003. Cyclooxygenase-2 expression does not correlate with outcome in osteosarcoma or rhabdomyosarcoma. Journal of Pediatric Hematology/Oncology 25, 282–285.

Dickerson, M.E., Page, R.L., LaDue, T.A., Hauck, M.L., Thrall, D.E., Stebbins, M.E., Price, G.S., 2001. Retrospective analysis of axial skeleton osteosarcoma in 22 large-breed dogs. Journal of Veterinary Internal Medicine 15, 120–124.

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. Human Gene Therapy 16, 937–946.

Ehrhart, N., Dernell, W.S., Hoffmann, W.E., Weigel, R.M., Powers, B.E., Withrow, S.J., 1998. Prognostic importance of alkaline phosphatase activity in serum from dogs with appendicular osteosarcoma: 75 cases (1990–1996). Journal of the American Veterinary Medical Association 213, 1002–1006.

Evans, L.B., 1983. Osteosarcoma in a young Great Dane dog. Journal of the South African Veterinary Association 54, 271–273.

Fan, T.M., de Lorimier, L.P., O'Dell-Anderson, K., Lacoste, H.I., Charney, S.C., 2007. Single-agent pamidronate for palliative therapy of canine appendicular osteosarcoma bone pain. Journal of Veterinary Internal Medicine 21, 431–439.

Fan, T.M., Charney, S.C., de Lorimier, L.P., Garrett, L.D., Griffon, D.J., Gordon-Evans, W.J., Wypij, J.M., 2009. Double-blind placebo-controlled trial of adjuvant pamidronate with palliative radiotherapy and intravenous doxorubicin for canine appendicular osteosarcoma bone pain. Journal of Veterinary Internal Medicine 23, 152–160.

Fernandis, A.Z., Wenk, M.R., 2009. Lipid-based biomarkers for cancer. Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences 877, 2830–2835.

Ferracini, R., Angelini, P., Cagliero, E., Linari, A., Martano, M., Wunder, J., Buracco, P., 2000. MET oncogene aberrant expression in canine osteosarcoma. Journal of Orthopaedic Research 18, 253–256.

Fieten, H., Spee, B., Ijzer, J., Kik, M.J., Penning, L.C., Kirpensteijn, J., 2009. Expression of hepatocyte growth factor and the proto-oncogenic receptor c-Met in canine osteosarcoma. Veterinary Pathology 46, 869–877.

Forrest, L.J., Dodge, R.K., Page, R.L., Heidner, G.L., McEntee, M.C., Novotney, C.A., Thrall, D.E., 1992. Relationship between quantitative tumor scintigraphy and time to metastasis in dogs with osteosarcoma. Journal of Nuclear Medicine 33, 1542–1547.

Foukas, A.F., Deshmukh, N.S., Grimer, R.J., Mangham, D.C., Mangos, E.G., Taylor, S., 2002. Stage-IIB osteosarcomas around the knee. A study of MMP-9 in surviving tumour cells. The Journal of Bone and Joint Surgery 84, 706–711.

Garzotto, C.K., Berg, J., Hoffmann, W.E., Rand, W.M., 2000. Prognostic significance of serum alkaline phosphatase activity in canine appendicular osteosarcoma. Journal of Veterinary Internal Medicine 14, 587–592.

Gogas, H., Eggermont, A.M., Hauschild, A., Hersey, P., Mohr, P., Schadendorf, D., Spatz, A., Dummer, R., 2009. Biomarkers in melanoma. Annals of Oncology 20, 8–13.

Gorman, E., Barger, A.M., Wypij, J.M., Pinkerton, M.E., 2006. Cutaneous metastasis of primary appendicular osteosarcoma in a dog. Veterinary Clinical Pathology 35, 358–361.

Hammer, A.S., Weeren, F.R., Weisbrode, S.E., Padgett, S.L., 1995. Prognostic factors in dogs with osteosarcomas of the flat or irregular bones. Journal of the American Animal Hospital Association 31, 321–326.

Hillers, K.R., Dernell, W.S., Lafferty, M.H., Withrow, S.J., Lana, S.E., 2005. Incidence and prognostic importance of lymph node metastases in dogs with appendicular osteosarcoma: 228 cases (1986–2003). Journal of the American Veterinary Medical Association 226, 1364–1367.

Hunter, K.W., 2004. Ezrin, a key component in tumor metastasis. Trends in Molecular Medicine 10, 201–204.

Johnson, A.S., Couto, C.G., Weghorst, C.M., 1998. Mutation of the p53 tumor suppressor gene in spontaneously occurring osteosarcomas of the dog. Carcinogenesis 19, 213–217.

Kaya, M., Wada, T., Akatsuka, T., Kawaguchi, S., Nagoya, S., Shindoh, M., Higashino, F., Mezawa, F., Okada, F., Ishii, S., 2000. Vascular endothelial growth factor expression in untreated osteosarcoma is predictive of pulmonary metastasis and poor prognosis. Clinical Cancer Research 6, 572–577.

Khanna, C., Wan, X., Bose, S., Cassaday, R., Olomu, O., Mendoza, A., Yeung, C., Gorlick, R., Hewitt, S.M., Helman, L.J., 2004. The membrane–cytoskeleton linker ezrin is necessary for osteosarcoma metastasis. Nature Medicine 10, 182–186.

Kim, M.S., Cho, W.H., Song, W.S., Lee, S.Y., Jeon, D.G., 2007. Time dependency of prognostic factors in patients with stage II osteosarcomas. Clinical Orthopaedics and Related Research 463, 157–165.

Kim, C., Shin, E., Hong, S., Chon, H.J., Kim, H.R., Ahn, J.R., Hong, M.H., Yang, W.I., Roh, J.K., Rha, S.Y., 2009a. Clinical value of ezrin expression in primary osteosarcoma. Cancer Research and Treatment 41, 138–144.

Kim, K., Aronov, P., Zakharkin, S.O., Anderson, D., Perroud, B., Thompson, I.M., Weiss, R.H., 2009b. Urine metabolomics analysis for kidney cancer detection and biomarker discovery. Molecular and Cellular Proteomics 8, 558–570.

Kirpensteijn, J., Kik, M., Rutteman, G.R., Teske, E., 2002. Prognostic significance of a new histologic grading system for canine osteosarcoma. Veterinary Pathology 39, 240–246.

Kirpensteijn, J., Kik, M., Teske, E., Rutteman, G.R., 2008. TP53 gene mutations in canine osteosarcoma. Veterinary Surgery 37, 454–460.

Kuntz, C.A., Dernell, W.S., Powers, B.E., Withrow, S., 1998. Extraskeletal osteosarcomas in dogs: 14 cases. Journal of the American Animal Hospital Association 34, 26–30.

Lacoste, H., Fan, T.M., de Lorimier, L.P., Charney, S.C., 2006. Urine N-telopeptide excretion in dogs with appendicular osteosarcoma. Journal of Veterinary Internal Medicine 20, 335–341.

Lana, S.E., Ogilvie, G.K., Hansen, R.A., Powers, B.E., Dernell, W.S., Withrow, S.J., 2000. Identification of matrix metalloproteinases in canine neoplastic tissue. American Journal of Veterinary Research 61, 111–114.

Langenbach, A., Anderson, M.A., Dambach, D.M., Sorenmo, K.U., Shofer, F.D., 1998. Extraskeletal osteosarcomas in dogs: a retrospective study of 169 cases (1986–1996). Journal of the American Animal Hospital Association 34, 113–120.

Lascelles, B.D., Dernell, W.S., Correa, M.T., Lafferty, M., Devitt, C.M., Kuntz, C.A., Straw, R.C., Withrow, S.J., 2005. Improved survival associated with postoperative wound infection in dogs treated with limb-salvage surgery for osteosarcoma. Annals of Surgical Oncology 12, 1073–1083.

Levine, R.A., Forest, T., Smith, C., 2002. Tumor suppressor PTEN is mutated in canine osteosarcoma cell lines and tumors. Veterinary Pathology 39, 372–378.

Liptak, J.M., Dernell, W.S., Straw, R.C., Rizzo, S.A., Lafferty, M.H., Withrow, S.J., 2004. Proximal radial and distal humeral osteosarcoma in 12 dogs. Journal of the American Animal Hospital Association 40, 461–467.

Loukopoulos, P., Robinson, W.F., 2007. Clinicopathological relevance of tumour grading in canine osteosarcoma. Journal of Comparative Pathology 136, 65–73.

Loukopoulos, P., Thornton, J.R., Robinson, W.F., 2003. Clinical and pathologic relevance of p53 index in canine osseous tumors. Veterinary Pathology 40, 237–248.

Loukopoulos, P., O'Brien, T., Ghoddusi, M., Mungall, B.A., Robinson, W.F., 2004. Characterisation of three novel canine osteosarcoma cell lines producing high levels of matrix metalloproteinases. Research in Veterinary Science 77, 131–141.

Lucas, P.W., Fan, T.M., Garrett, L.D., Griffon, D.J., Wypij, J.M., 2008. A comparison of five different bone resorption markers in osteosarcoma-bearing dogs, normal dogs, and dogs with orthopedic diseases. Journal of Veterinary Internal Medicine 22, 1008–1013.

MacEwen, E.G., Kutzke, J., Carew, J., Pastor, J., Schmidt, J.A., Tsan, R., Thamm, D.H., Radinsky, R., 2003. C-Met tyrosine kinase receptor expression and function in human and canine osteosarcoma cells. Clinical and Experimental Metastasis 20, 421–430.

McNeill, C.J., Overley, B., Shofer, F.S., Kent, M.S., Clifford, C.A., Samluk, M., Haney, S., Van Winkle, T.J., Sorenmo, K.U., 2007. Characterization of the biological behaviour of appendicular osteosarcoma in Rottweilers and a comparison with other breeds: a review of 258 dogs. Veterinary and Comparative Oncology 5, 90–98.

Misdorp, W., Hart, A.A., 1979. Some prognostic and epidemiologic factors in canine osteosarcoma. Journal of the National Cancer Institute 62, 537–545.

Mohammed, S.I., Khan, K.N., Sellers, R.S., Hayek, M.G., DeNicola, D.B., Wu, L., Bonney, P.L., Knapp, D.W., 2004. Expression of cyclooxygenase-1 and 2 in naturally-occurring canine cancer. Prostaglandins, Leukotrienes, and Essential Fatty Acids 70, 479–483.

Moore, A.S., Dernell, W.S., Ogilvie, G.K., Kristal, O., Elmslie, R., Kitchell, B., Susaneck, S., Rosenthal, R., Klein, M.K., Obradovich, J., Legendre, A., Haddad, T., Hahn, K., Powers, B.E., Warren, D., 2007. Doxorubicin and BAY 12-9566 for the treatment of osteosarcoma in dogs: a randomized, double-blind, placebo-controlled study. Journal of Veterinary Internal Medicine 21, 783–790.

Mullins, M.N., Lana, S.E., Dernell, W.S., Ogilvie, G.K., Withrow, S.J., Ehrhart, E.J., 2004. Cyclooxygenase-2 expression in canine appendicular osteosarcomas. Journal of Veterinary Internal Medicine 18, 859–865.

Nakano, T., Tani, M., Ishibashi, Y., Kimura, K., Park, Y.B., Imaizumi, N., Tsuda, H., Aoyagi, K., Sasaki, H., Ohwada, S., Yokota, J., 2003. Biological properties and gene expression associated with metastatic potential of human osteosarcoma. Clinical and Experimental Metastasis 20, 665–674.

Norrdin, R.W., Powers, B.E., Torgersen, J.L., Smith, R.E., Withrow, S.J., 1989. Characterization of osteosarcoma cells from two sibling large-breed dogs. American Journal of Veterinary Research 50, 1971–1975.

O'Brien, M.G., Straw, R.C., Withrow, S.J., Powers, B.E., Jameson, V.J., Lafferty, M., Ogilvie, G.K., LaRue, S.M., 1993. Resection of pulmonary metastases in canine osteosarcoma: 36 cases (1983–1992). Veterinary Surgery 22, 105–109.

Owen, L.N., 1967. Comparative aspects of bone tumours in man and dog. Proceedings of the Royal Society of Medicine 60, 1309–1310.

Page, R.L., Hughes, C.S., Huyan, S., Sagris, J., Trogdon, M., 2000. Modulation of P-glycoprotein-mediated doxorubicin resistance in canine cell lines. Anticancer Research 20, 3533–3538.

Pakos, E.E., Nearchou, A.D., Grimer, R.J., Koumoullis, H.D., Abudu, A., Bramer, J.A., Jeys, L.M., Franchi, A., Scoccianti, G., Campanacci, D., Capanna, R., Aparicio, J., Tabone, M.D., Holzer, G., Abdolvahab, F., Funovics, P., Dominkus, M., Ilhan, I., Berrak, S.G., Patino-Garcia, A., Sierrasesumaga, L., San-Julian, M., Garraus, M., Petrilli, A.S., Filho, R.J., Macedo, C.R., Alves, M.T., Seiwerth, S., Nagarajan, R., Cripe, T.P., Ioannidis, J.P., 2009. Prognostic factors and outcomes for osteosarcoma: an international collaboration. European Journal of Cancer 45, 2367–2375.

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.

Peremans, K., Otte, A., Verschooten, F., Van Bree, H., Dierckx, R., 2003. Soft tissue metastasis of an osteosarcoma of the humerus in a four-legged patient. European Journal of Nuclear Medicine and Molecular Imaging 30, 188.

Phillips, B., Powers, B.E., Dernell, W.S., Straw, R.C., Khanna, C., Hogge, G.S., Vail, D.M., 2009. Use of single-agent carboplatin as adjuvant or neoadjuvant therapy in conjunction with amputation for appendicular osteosarcoma in dogs. Journal of the American Animal Hospital Association 45, 33–38.

Powers, B.E., Withrow, S.J., Thrall, D.E., Straw, R.C., LaRue, S.M., Page, R.L., Gillette, E.L., 1991. Percent tumor necrosis as a predictor of treatment response in canine osteosarcoma. Cancer 67, 126–134.

Rodriguez, N.I., Hoots, W.K., Koshkina, N.V., Morales-Arias, J.A., Arndt, C.A., Inwards, C.Y., Hawkins, D.S., Munsell, M.F., Kleinerman, E.S., 2008. COX-2 expression correlates with survival in patients with osteosarcoma lung metastases. Journal of Pediatric Hematology/Oncology 30, 507–512.

Ru, G., Terracini, B., Glickman, L.T., 1998. Host related risk factors for canine osteosarcoma. Veterinary Journal 156, 31–39.

Sagartz, J.E., Bodley, W.L., Gamblin, R.M., Couto, C.G., Tierney, L.A., Capen, C.C., 1996. P53 tumor suppressor protein overexpression in osteogenic tumors of dogs. Veterinary Pathology 33, 213–221.

Salas, S., de Pinieux, G., Gomez-Brouchet, A., Larrousserie, F., Leroy, X., Aubert, S., Decouvelaere, A.V., Giorgi, R., Fernandez, C., Bouvier, C., 2009. Ezrin immunohistochemical expression in cartilaginous tumours: a useful tool for differential diagnosis between chondroblastic osteosarcoma and chondrosarcoma. Virchows Archiv 454, 81–87.

Selting, K.A., Lana, S.E., Ogilvie, G.K., Olmstead, A., Mykles, D.L., Bright, J., Richardson, K.L., Walton, J.A., Monnet, E., Fettman, M.J., 2004. Cardiac troponin I in canine patients with lymphoma and osteosarcoma receiving doxorubicin: comparison with clinical heart disease in a retrospective analysis. Veterinary and Comparative Oncology 2, 142–156.

Selvarajah, G.T., Kirpensteijn, J., van Wolferen, M.E., Rao, N.A., Fieten, H., Mol, J.A., 2009. Gene expression profiling of canine osteosarcoma reveals genes associated with short and long survival times. Molecular Cancer 8, 72.

Spodnick, G.J., Berg, J., Rand, W.M., Schelling, S.H., Couto, G., Harvey, H.J., Henderson, R.A., MacEwen, G., Mauldin, N., McCaw, D.L., et al., 1992. Prognosis for dogs with appendicular osteosarcoma treated by amputation alone: 162 cases (1978–1988). Journal of the American Veterinary Medical Association 200, 995–999.

Srivastava, A., Fuchs, B., Zhang, K., Ruan, M., Halder, C., Mahlum, E., Weber, K., Bolander, M.E., Sarkar, G., 2006. High WT1 expression is associated with very poor survival of patients with osteogenic sarcoma metastasis. Clinical Cancer Research 12, 4237–4243.

Straw, R.C., Withrow, S.J., 1996. Limb-sparing surgery versus amputation for dogs with bone tumors. The Veterinary Clinics of North America. Small Animal Practice 26, 135–143.

Straw, R.C., Powers, B.E., Klausner, J., Henderson, R.A., Morrison, W.B., McCaw, D.L., Harvey, H.J., Jacobs, R.M., Berg, R.J., 1996. Canine mandibular osteosarcoma: 51 cases (1980–1992). Journal of the American Animal Hospital Association 32, 257–262.

Thamm, D.H., O'Brien, M.G., Vail, D.M., 2008. Serum vascular endothelial growth factor concentrations and postsurgical outcome in dogs with osteosarcoma. Veterinary and Comparative Oncology 6, 126–132.

Thomas, R., Scott, A., Langford, C.F., Fosmire, S.P., Jubala, C.M., Lorentzen, T.D., Hitte, C., Karlsson, E.K., Kirkness, E., Ostrander, E.A., Galibert, F., Lindblad-Toh, K., Modiano, J.F., Breen, M., 2005. Construction of a 2-Mb resolution BAC microarray for CGH analysis of canine tumors. Genome Research 15, 1831–1837.

Tomlin, J.L., Sturgeon, C., Pead, M.J., Muir, P., 2000. Use of the bisphosphonate drug alendronate for palliative management of osteosarcoma in two dogs. Veterinary Record 147, 129–132.

Uchibori, M., Nishida, Y., Nagasaka, T., Yamada, Y., Nakanishi, K., Ishiguro, N., 2006. Increased expression of membrane-type matrix metalloproteinase-1 is

correlated with poor prognosis in patients with osteosarcoma. International Journal of Oncology 28, 33–42.
Urakawa, H., Nishida, Y., Naruse, T., Nakashima, H., Ishiguro, N., 2009. Cyclooxygenase-2 overexpression predicts poor survival in patients with high-grade extremity osteosarcoma: a pilot study. Clinical Orthopaedics and Related Research 467, 2932–2938.
Vail, D.M., Kurzman, I.D., Glawe, P.C., O'Brien, M.G., Chun, R., Garrett, L.D., Obradovich, J.E., Fred 3rd, R.M., Khanna, C., Colbern, G.T., Working, P.K., 2002. STEALTH liposome-encapsulated cisplatin (SPI-77) versus carboplatin as adjuvant therapy for spontaneously arising osteosarcoma (OSA) in the dog: a randomized multicenter clinical trial. Cancer Chemotherapy and Pharmacology 50, 131–136.
van Leeuwen, I.S., Cornelisse, C.J., Misdorp, W., Goedegebuure, S.A., Kirpensteijn, J., Rutteman, G.R., 1997. P53 gene mutations in osteosarcomas in the dog. Cancer Letters 111, 173–178.
Vousden, K.H., 2002. Activation of the p53 tumor suppressor protein. Biochimica et Biophysica Acta 1602, 47–59.
Walter, C.U., Dernell, W.S., LaRue, S.M., Lana, S.E., Lafferty, M.H., LaDue, T.A., Withrow, S.J., 2005. Curative-intent radiation therapy as a treatment modality for appendicular and axial osteosarcoma: a preliminary retrospective evaluation of 14 dogs with the disease. Veterinary and Comparative Oncology 3, 1–7.
Walters, D.K., Steinmann, P., Langsam, B., Schmutz, S., Born, W., Fuchs, B., 2008. Identification of potential chemoresistance genes in osteosarcoma. Anticancer Research 28, 673–679.
Wenk, M.R., 2005. The emerging field of lipidomics. Nature Reviews Drug Discovery 4, 594–610.
Wergin, M.C., Kaser-Hotz, B., 2004. Plasma vascular endothelial growth factor (VEGF) measured in seventy dogs with spontaneously occurring tumours. In Vivo 18, 15–19.
Xu-Dong, S., Zan, S., Shui-er, Z., Li-na, T., Wen-xi, Y., Feng, L., Yang, Y., 2009. Expression of Ezrin correlates with lung metastasis in Chinese patients with osteosarcoma. Clinical and Investigative Medicine 32, E180–188.