博士論文(要約)

The anti-tumour effects of etoposide on canine osteosarcoma

(犬骨肉腫に対するエトポシドの抗腫瘍効果)

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平成 25 年度入学

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Contents

General introduction

Osteosarcoma (OSA) is the most common primary sarcoma of bone. It accounts for 80 – 90% of canine primary bone neoplasms where large- and giant breed dogs are at greater risk of developing this disease (Ru *et al.*, 1998; Morris and Dobson, 2001; McNeill *et al.*, 2007; Rosenberger *et al.*, 2007). Canine OSA tends to occur in older male dogs, though some reports showed an increased incidence in dogs less than 2 years of age (Misdorp and Hart, 1979; Mauldin *et al.*, 1988; Spodnick *et al.*, 1992; Boston *et al.*, 2006; McNeill *et al.*, 2007; Rosenberger *et al.*, 2007). Several studies have suggested that neutered status is associated with the development of OSA, with neutered dogs being more predisposed to the disease than intact dogs (Ru *et al.*, 1998; Cooley *et al.*, 2002).

The locally invasive nature of canine OSA requires a wide marginal excision by amputation or limb salvage procedures, which are the most effective means of treatment for the primary tumour and pain relief (Mauldin *et al.*, 1988; Szewczyk *et al.*, 2014). However, the major dilemma of the canine OSA treatment is the presence of micrometastases at the time of presentation or diagnosis. More than 70% of canine OSA patients are euthanized or died due to metastases with only 11 – 21% of the dogs are alive at 1 year post-surgery when treated with surgery alone (Brodey and Abt, 1976; Mauldin *et al.*, 1988; Spodnick *et al.*, 1992). Some reports suggested that both human and canine OSA patients benefit from aggressive surgical resection of pulmonary metastases; however, more studies are needed to determine the clinical advantage of this procedure over the operative and post-operative complications (Snyder *et al.*, 1991; O'brien *et al.*, 1993).

Combination of surgery and adjuvant chemotherapy using doxorubicin, cisplatin, or carboplatin extends survival rate of dogs with OSA to 35 – 50% at 1 year. Yet, adjuvant chemotherapy fails to impede the progression of the metastatic disease,

which is the ultimate cause of death (Straw *et al.*, 1991; Berg *et al.*, 1992, 1995; Bergman *et al.*, 1996; Boston *et al.*, 2006; Moore *et al.*, 2007; Bacon *et al.*, 2008; Phillips *et al.*, 2009; Oblak *et al.*, 2012; Szewczyk *et al.*, 2014). The anti-neoplastic agents mentioned above are extensively used in both human and veterinary oncology and are part of the treatment protocol of a variety of cancers. Nonetheless, each drug is associated with factors that limit its use. It has been proven that doxorubicin is capable of inducing cardiomyopathy in dogs, which is often fatal; while cisplatin administration is associated with nephrotoxicity, restricting their use in patients with cardiovascular disease and renal impairment, respectively (Daugaard *et al.*, 1987; Mauldin *et al.*, 1992; Berg *et al.*, 1995; McDuffie *et al.*, 2010; Gallay-Lepoutre *et al.*, 2016). The safer toxicity profile of carboplatin makes it a better candidate for canine OSA treatment. However, the high cost of carboplatin alongside canine OSA being a disease that commonly affects large- and giant-breed dogs render treatments to be costly.

Various novel anti-cancer therapies have been developed in recent years, these include molecular targeted drugs, immunotherapy and nanoparticles. Despite that, no breakthrough has yet been achieved in canine OSA treatment and the prognosis of canine OSA remains universally poor (MacEwen *et al.*, 1989; Vail *et al.*, 2002; Selvarajah and Kirpensteijn, 2010). The treatment options for canine OSA are limited and most new therapeutic approaches are still under investigation or commercially unavailable. Furthermore, research and development of new drugs are time-consuming and costly. Therefore, there is a clinical need for alternative treatment options or drug-repositioning that are effective, safe and affordable using drugs that are readily available.

From a preliminary study conducted to evaluate the sensitivity of canine OSA, mammary gland carcinoma (MGT) and melanoma cell lines to several topoisomerase inhibitors, canine OSA cell lines exhibited higher sensitivity than MGT and melanoma cell lines to all the topoisomerase inhibitors tested *in vitro*, including etoposide. In addition, etoposide treatment markedly delayed tumour progression in canine OSA xenografts but not the canine MGT xenografts. However, the treatment regimen employed in this study caused systemic toxicity and further study is necessary to refine the treatment protocol (Ong *et al.*, in press). Nevertheless, the findings presented in this study have opened interesting possibilities on the use of etoposide in veterinary oncology, especially for canine OSA.

Etoposide is a semisynthetic derivative of podophyllotoxin that disrupts DNA synthesis by inhibiting topoisomerase II and consequently cause DNA double strand breaks (Gantchev and Hunting, 1997). It has proven efficacy for a wide range of neoplasms including small cell lung cancer, testicular cancers, Hodgkin's and non-Hodgkin's lymphomas, and acute leukaemia in humans (Hande, 1998; Gerritsen-van Schieveen *et al.*, 2011). It has also been employed in some human OSA treatment protocols with varying outcomes (Le Deley *et al.*, 2007; O'Kane *et al.*, 2015; Whelan *et al.*, 2015; Schwartz *et al.*, 2016). Previous clinical trials on recurrent canine lymphoma and hemangiosarcoma have demonstrated that etoposide, either administered alone or in combination with other agents, may be a potential anti-neoplastic agent for clinical application in veterinary oncology (Hohenhaus and Matus, 1990; Lana *et al.*, 2007).

For the past decades, rapid advances in cancer research revealed that dynamic changes in the genome are attributed to development of cancer. The paths involved in oncogenesis are highly variable and frequently multigenic. Consequently, anti-cancer

therapies utilizing single-targeted drugs may not be able to sufficiently cure a complex disease such as cancer and multi-targeted therapies or combinational therapies are required to counter this condition (Hanahan and Weinberg, 2000; Zimmermann *et al.*, 2007). It has been suggested that anti-cancer therapy using combination of drugs with different cytotoxic mechanisms and less overlapping toxicity can achieve better response rate that monotherapy (Yamanaka *et al.*, 2011). Hence, it is appealing to evaluate the effects of etoposide in combination with other anti-cancer compounds on canine OSA.

The aims of this study were to investigate the anti-tumour efficacy of etoposide on canine OSA, to identify novel combination therapeutic regimen for the treatment of canine OSA and its mechanism of action, and to establish a treatment regimen applicable in clinical setting. In chapter 1, the anti-tumour effects of etoposide alone or in combination with a non-steroidal anti-inflammatory drug, piroxicam, on canine OSA cell lines were evaluated. The mechanism of actions and changes in the molecular machinery that contributed to the anti-tumour effects were elucidated. Experiments in chapter 2 were conducted based on the findings in chapter 1, where the synergistic anti-tumour effects exhibited by combination treatment of etoposide and piroxicam was deduced to be associated with survivin down-regulation. However, these improved effects were only apparent at the concentrations of piroxicam that exceed clinically attainable concentration. Thus, the anti-tumour effects of etoposide in combination with a survivin inhibitor (YM155) were explored in chapter 2. Finally, chapter 3 consists of the *in vivo* studies on the efficacy of etoposide therapy either alone or in combination with either piroxicam or YM155 using canine OSA xenograft mouse models.

Chapter 1

Cytotoxic effects of etoposide alone or in combination with piroxicam on canine OSA cell lines

The contents of this chapter have been published in The Veterinary Journal vol. 218, pp. 51-59 in Dec 2016.

Chapter 2

Cytotoxic effects of etoposide alone or in combination with a survivin inhibitor, YM155, on canine osteosarcoma cell lines

The contents in this chapter are scheduled to be published as part of a journal.

Chapter 3

Anti-tumour effects of etoposide alone or in combination with either piroxicam or YM155 on canine osteosarcoma xenografts

Introduction

It has been universally acknowledged that NSAIDs have the capability to hamper tumorigenesis by inhibiting the production of prostaglandin, which is essential for tumour formation including transitional cell carcinoma, colorectal neoplasms, mammary gland carcinoma, squamous cell carcinoma and OSA (Mohammed *et al.*, 1999; Fosslien, 2000; Schmidt *et al.*, 2001; Mullins *et al.*, 2004). Besides that, various evidence show NSAIDs inhibit cancer cell proliferation, induce apoptosis and sensitize tumour cells to chemotherapeutic agents (Hanif *et al.*, 1996; Knapp *et al.*, 2000; Grösch *et al.*, 2001; Mohammed *et al.*, 2002). In chapter 1 of this study, it has been demonstrated that piroxicam enhanced the cytotoxic effect of etoposide, which interferes with the surveillance machinery of DNA leading to DNA double strand breaks and subsequently triggers the apoptotic cascades in canine OSA cells *in vitro*, but at concentrations that exceed the clinically achievable concentration.

Additionally, combination of etoposide with YM155 also exhibited superior anti-proliferative activity than either drug alone in chapter 2. YM155 is a promising novel anti-neoplastic compound that selectively blocks the expression of survivin protein, which is predominantly expressed in cancer cells. Suppression of survivin by YM155 reduces the threshold of apoptosis and sensitizes cancer cells to cytotoxic agents (Arora *et al.*, 2012; Nakahara *et al.*, 2011; Yamanaka *et al.*, 2011; Dresang *et al.*, 2013; Mir *et al.*, 2014; Yamazaki *et al.*, 2015; Zhang, Z. *et al.*, 2015). Although numerous studies showed that the improved outcome of concomitant treatment with conventional anti-neoplastic agent and YM155 was a result of augmented apoptosis, the findings in chapter 2 suggest that the cytotoxic mechanism was not universally identical.

Nonetheless, studies in the previous chapters were conducted on cultured cell. There are some limitations in *in vitro* studies as cancer cells are kept in an environment that is incapable to fully replicate the condition *in vivo*. Some drugs, such as NSAIDs, exert their anti-tumour effects by modulating the tumour microenvironment, immune system or circulatory system that are absent in cell culture studies. Knapp *et al.* demonstrated that piroxicam improved the remission rate in canine patients with transitional cell carcinoma at concentration that was non-cytotoxic *in vitro,* signifying results from cell culture analyses may not reflect the outcome *in vivo* precisely. On top of that, pharmacokinetic optimisation is essential for novel drug or drug combination; thus, studies using animal models are inevitable.

Cell proliferation is a fundamental biological process controlled by organized mechanisms. Dysregulation of this meticulous program that results in persistent cell proliferation may cause tumour formation (Schlüter *et al.*, 1993). The course of tumour development is also dependent on the level of neoplastic cell death. Compensatory suppression of apoptosis, which is essential to constrain growth in normal tissue and to eliminate genetically aberrant cells, supports neoplastic progression (Thompson, 1995; Evan and Vousden, 2001). Therefore, tumourigenesis is imminent when there are disruptions in the cell proliferation and apoptosis regulatory machineries. Since the defects in these two mechanisms are the cores of tumour establishment, they serve as important targets for therapeutic interventions and assessment of therapeutic efficacy (Evan and Vousden, 2001).

The in vivo anti-tumour efficacy of etoposide against canine OSA has been reported previously, but the treatment regimen of the study resulted in systemic toxicity (Ong *et al.*, in press). Therefore, the purpose of this chapter was to investigate the effectiveness of etoposide treatment alone and in combination with piroxicam

(section 1) or YM155 (section 2) against canine OSA using xenograft mouse models and establish a suitable treatment protocol that could be translated into clinical application. The anti-tumour efficacy of each regimen was evaluated in the aspects of tumour growth, cell proliferation and apoptosis.

The contents in this chapter are scheduled to be published as part of a journal.

Summary and Conclusion

Canine OSA is an aggressive bone neoplasm with more than 90% of the patients have micrometastases at the time of diagnosis. Hence, most patients succumb to the disease despite removal of the primary tumour via amputation or limb-sparing surgery within several months. The use of adjuvant chemotherapy, such as doxorubicin, carboplatin and cisplatin, in patients that underwent surgical removal of the primary mass prolonged overall survival. However, these patients eventually suffer from metastases, which is the ultimate cause of death. Recently, there have been great advances in oncology research that provided in-depth understanding of tumour biology and aid the development of novel cancer therapeutics. For years, efforts to improve the survival outcome of canine OSA patients remain futile without major breakthrough in its anti-cancer therapy. Besides that, a lot of potential novel treatments are still in the research stage and would take a long time to be available commercially. These highlight an urgent need for novel treatment approach for canine OSA using readily available drugs.

Findings from a preliminary study revealed canine OSA cell lines were sensitive to topoisomerase inhibitors, which included etoposide, both *in vitro* and *in vivo*. Etoposide has been employed in the treatment protocol for human OSA, and since OSA in dogs is similar to humans in various aspects, further investigation on etoposide as a potential chemotherapeutic agent for veterinary application is warranted. Besides, multi-targeted therapy may be attractive, as it has been suggested to deliver better response rate than single-targeted therapy. Therefore, the objectives of this study were to investigate the anti-tumour efficacy of etoposide on canine OSA and identify novel combination therapeutic regimen for this disease. Furthermore, the effect of treatment on the underlying molecular machineries was elucidated to gain better insight into the anti-tumour mechanisms.

In the first chapter, the cytotoxic effects of etoposide alone and in combination with piroxicam on canine OSA cell lines were examined. All canine OSA cell lines were sensitive to etoposide treatment at 0.2 μM, which is a concentration attainable *in vivo*. Etoposide induced cells to undergo G_2/M arrest through inactivation of the Cdc2-cyclin B1 complex and subsequently triggered the apoptosis cascade. Whilst piroxicam alone did not show anti-proliferative effect, combination treatment with etoposide and piroxicam demonstrated better cytotoxic effect than either drug alone. Piroxicam chemosensitized all OSA cell lines to the inhibitory effect of etoposide, and augmented the proportion of G_2/M and apoptotic cells. Intriguingly, analyses of the cellular proteins revealed that one of the underlying mechanisms that contributed to this synergistic effect was down-regulation of the expression of survivin, which is an IAP, and subsequently up-regulation of cleaved PARP.

Of note, these enhanced effects were evident only when piroxicam was added at concentrations higher than the physiological achievable concentration and it was postulated that these synergistic anti-proliferative effect might be absent *in vivo*. However, cell culture experiments are unable to fully replicate the condition *in vivo*; thus, further investigation using animal models was performed in chapter 3 to validate the anti-tumour efficacy of etoposide alone and in combination with piroxicam against canine OSA. Since suppression of survivin was associated with enhanced cytotoxicity in combination treatment, adjunct treatment with a survivin inhibitor may exhibit comparable inhibitory effects on canine OSA.

Therefore, the study on the effects of etoposide in combination with a survivin inhibitor molecule, YM155, on canine OSA cell lines was conducted and presented in chapter 2. The IC_{50} of YM155 for each canine OSA cell line was at nanomolar concentration, which is within or below the canine or murine serum concentration.

YM155 enhanced the cytotoxicity of etoposide on all canine OSA cell lines, but the effect of treatment on cell cycle regulatory machineries was heterogenous. Unlike the previous drug combination, etoposide together with YM155 did not induce G_2/M arrest nor inactivation of the Cdc2-cyclin B1 complex, but promoted accumulation of cells in S phase and reduction of G1 fraction in POS and HOS cell lines, and had little effect on the cell cycle distribution of HMPOS cell line.

Survivin knock down by YM155 is known to sensitise cancer cells to chemotherapy-induced apoptosis. Surprisingly, combination treatment with etoposide and YM155 enhanced the apoptotic activity of HOS cell line only. It remains unclear in what way YM155 enhanced the inhibitory effect of etoposide on HMPOS and POS cell lines, but it was speculated that YM155 might target other signalling molecules besides survivin. Nonetheless, given the apparent synergistic effects demonstrated by this therapeutic combination, further study *in vivo* was conducted to determine a suitable treatment protocol and evaluate the anti-tumour efficacy and safety profile of this drug combination.

Next, the anti-neoplastic efficacy of etoposide alone and in combination with piroxicam was evaluated using xenograft mouse models in the first section of chapter 3. Both etoposide single agent treatment and combination treatment with piroxicam delayed xenograft tumour progression predominantly through suppression of tumour cell proliferation. While the protein expression of survivin was reduced in these 2 groups, it was not accompanied with an increase in apoptotic activity, which is contrary to the findings in cell culture study. This could be due to the interaction between cancer cells with certain ECM compounds that modified their response to anti-neoplastic agents and blocked the apoptosis cascade. In agreement with the hypothesis postulated, addition of piroxicam into etoposide treatment regime did not

further suppress tumour growth when compared with etoposide single agent treatment. Meanwhile, piroxicam single agent treatment failed to suppress xenograft tumour growth. Considering canine OSA is commonly associated with pain, and incorporation of piroxicam into etoposide treatment regimen did not cause adverse reaction, this therapeutic combination is a promising alternative therapy for canine OSA.

In the second section of chapter 3, the *in vivo* anti-tumour efficacy of etoposide in combination with YM155 was evaluated. Although statistically insignificant, combination treatment reduced the growth rate of the xenograft tumours by 70%, primarily through alteration of the cell proliferation machineries. Surprisingly, the anti-neoplastic effect exhibited by etoposide and YM155 single agent treatment was negligible. Besides, survivin expression was not suppressed following administration of YM155, indicating further study is needed to improve the treatment protocol. It is hypothesised that the anti-tumour effect would be aggravated following optimisation of the treatment regimen. Taken together, these findings suggest that concomitant treatment with etoposide and YM155 is a potential novel therapeutic approach for canine OSA, but further improvement of the treatment regimen is necessary before proceeding to clinical trial.

In conclusion, this study revealed that etoposide exhibits effective anti-neoplastic activity against canine OSA both *in vitro* and *in vivo*. Addition of piroxicam into etoposide therapy does not improve the anti-tumour effect of etoposide, but this combination therapeutic regimen is feasible as it does not cause severe adverse reaction and may help alleviate cancer pain; thus, improving the life quality of canine OSA patients. Concomitant treatment with etoposide and a survivin inhibitor demonstrates superior anti-neoplastic effect than etoposide single agent

treatment, and this is a promising novel combination therapy for canine OSA. However, more work to optimise the treatment regimen is required. Although the anti-neoplastic mechanisms of etoposide treatment either alone or in combination with piroxicam or survivin inhibitor have not been completely elucidated, modification in the cell cycle progression machineries is one of the mechanisms involved.

Acknowledgement

First and foremost, I would like to express my deepest appreciation to my advisors, Prof. Ryohei Nishimura and Assoc. Prof. Takayuki Nakagawa, for their immense knowledge, patience, and persistent help in my Ph.D study. They have given me intellectual freedom in my work and endless opportunities to expand my professional network. Without their guidance and continuous support this dissertation would not have been possible.

Every experiment and result in this thesis was accomplished with the help of Dr. Kohei Saeki. His unwavering enthusiasm for science and continuous guidance has molded me into a better scientist. I greatly benefited from his scientific insight and immense knowledge in research. I am also grateful to Dr. Nan Choisunirachon who taught me the fundamental research techniques. Her hospitality has provided a friendly atmosphere in the laboratory.

Besides, I am indebted to Prof. Hiroyuki Nakayama, Laboratory of Veterinary Pathology, and Prof. Keiichiro Maeda, Laboratory of Theriogenology, The University of Tokyo, who gave access to their laboratory and research facilities. Otherwise, it would not be possible to conduct this research.

I extend my gratitude Dr. Naoki Fujita for his persistent efforts in maintaining the laboratory in an orderly manner. Without his efforts research would have undoubtedly been more difficult. I would also like to thank my fellow lab-mates for their precious comments and help in the last four years.

I am grateful for The Japanese Ministry of Education, Culture, Sports, Science and Technology, MEXT, for providing financial support for my graduate studies in The University of Tokyo.

Finally, I would like to thank my mother, brother, sister, Harley and Panther. This journey would not have been possible without their constant encouragement and spiritual support. I am especially grateful to Kok Mun Keong for his companionship, patience and unyielding support throughout my endeavor.

References

Adida, C., Haioun, C., Gaulard, P., Lepage, E., Morel, P., Briere, J., Dombret, H., Reyes, F., Diebold, J., Gisselbrecht, C. and Salles, G., 2000. Prognostic significance of survivin expression in diffuse large B-cell lymphomas. *Blood*, 96:1921-1925.

Altieri, D.C., 2003. Survivin, versatile modulation of cell division and apoptosis in cancer. *Oncogene*, 22:8581-8589.

Ambrosini, G., Adida, C. and Altieri, D.C., 1997. A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. *Nature medicine*, 3:917-921.

Arora, R., Shuda, M., Guastafierro, A., Feng, H., Toptan, T., Tolstov, Y., Normolle, D., Vollmer, L.L., Vogt, A., Dömling, A. and Brodsky, J.L., 2012. Survivin is a therapeutic target in Merkel cell carcinoma. *Science translational medicine*, 4:133ra56-133ra56.

Awara, W.M., El-Sisi, A.E., El-Sayad, M.E. and Goda, A.E., 2004. The potential role of cyclooxygenase-2 inhibitors in the treatment of experimentally-induced mammary tumour: does celecoxib enhance the anti-tumour activity of doxorubicin?. *Pharmacological research*, 50:487-498.

Bacon, N.J., Ehrhart, N.P., Dernell, W.S., Lafferty, M. and Withrow, S.J., 2008. Use of alternating administration of carboplatin and doxorubicin in dogs with microscopic metastases after amputation for appendicular osteosarcoma: 50 cases (1999–2006). *Journal of the American Veterinary Medical Association*, 232:1504-1510.

Barlogie, B. and Drewinko, B., 1978. Cell cycle stage-dependent induction of G2 phase arrest by different antitumor agents. *European Journal of Cancer (1965)*, 14:741-745.

Berg, J., Weinstein, M.J., Schelling, S.H. and Rand, W.M., 1992. Treatment of dogs with osteosarcoma by administration of cisplatin after amputation or limb-sparing surgery: 22 cases (1987-1990). *Journal of the American Veterinary Medical Association*, *200:*2005-2008. Abstract retrieved September 7, 2016 from Europe PMC.

Berg, J., Weinstein, M.J., Springfield, D.S. and Rand, W.M., 1995. Results of surgery and doxorubicin chemotherapy in dogs with osteosarcoma. *Journal of the American Veterinary Medical Association*, 206:1555-1560. Abstract retrieved September 7, 2016 from Europe PMC.

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. and Norris, A.M., 1996. Amputation and carboplatin for treatment of dogs with osteosarcoma: 48 cases (1991 to 1993). *Journal of Veterinary Internal Medicine*, 10:76-81.

Bongiovanni, L., Colombi, I., Fortunato, C. and Salda, L.D., 2009. Survivin expression in canine epidermis and in canine and human cutaneous squamous cell carcinomas. *Veterinary dermatology*, 20:369-376.

Bongiovanni, L., Mazzocchetti, F., Malatesta, D., Romanucci, M., Ciccarelli, A., Buracco, P., De Maria, R., Palmieri, C., Martano, M., Morello, E. and Maniscalco, L., 2012. Immunohistochemical investigation of cell cycle and apoptosis regulators (survivin, β-catenin, p53, caspase 3) in canine appendicular osteosarcoma. *BMC veterinary research*, 8:1.

Bongiovanni, L., Romanucci, M., Malatesta, D., D'Andrea, A., Ciccarelli, A. and Della Salda, L., 2015. Survivin and related proteins in canine mammary tumors immunohistochemical expression. *Veterinary Pathology,* 52:269-275.

Boston, S.E., Ehrhart, N.P., Dernell, W.S., Lafferty, M. and 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.

Brodey, R.S. and Abt, D.A., 1976. Results of surgical treatment in 65 dogs with osteosarcoma. *Journal of the American Veterinary Medical Association*, 168:1032-1035. Abstract retrieved February 16, 2016 from Europe PMC.

Brun, S.N., Markant, S.L., Esparza, L.A., Garcia, G., Terry, D., Huang, J.M., Pavlyukov, M.S., Li, X.N., Grant, G.A., Crawford, J.R. and Levy, M.L., 2015. Survivin as a therapeutic target in Sonic hedgehog-driven medulloblastoma. *Oncogene*, 34:3770-3779.

Caicedo-Granados, E.E., Wuertz, B.R., Marker, P.H., Lee, G.S. and Ondrey, F.G., 2011. The Effect of indomethacin on paclitaxel sensitivity and apoptosis in oral squamous carcinoma cells: the role of nuclear factor–κB Inhibition. *Archives of Otolaryngology–Head & Neck Surgery*, 137:799-805.

Chen, S.F., Zhang, Z.Y. and Zhang, J.L., 2015. Meloxicam increases intracellular accumulation of doxorubicin via downregulation of multidrug resistance-associated protein 1 (MRP1) in A549 cells. *Genetics and Molecular Research*, 14:14548-14560.

Cooley, D.M., Beranek, B.C., Schlittler, D.L., Glickman, N.W., Glickman, L.T. and Waters, D.J., 2002. Endogenous gonadal hormone exposure and bone sarcoma risk. *Cancer Epidemiology Biomarkers & Prevention*, 11:1434-1440.

Dai, D., Liang, Y., Xie, Z., Fu, J., Zhang, Y. and Zhang, Z., 2012. Survivin deficiency induces apoptosis and cell cycle arrest in HepG2 hepatocellular carcinoma cells. *Oncology reports*, 27:621-627.

Daugaard, G., Abildgaard, U., Larsen, S., Holstein-Rathlou, N.H., Amtorp, O., Olesen, H.P. and Leyssac, P.P., 1987. Functional and histopathological changes in dog kidneys after administration of cisplatin. *Kidney and Blood Pressure Research*, 10:54-64.

Davies, J., Heeb, H., Garimella, R., Templeton, K., Pinson, D. and Tawfik, O., 2012. Vitamin D receptor, retinoid X receptor, Ki-67, survivin, and ezrin expression in canine osteosarcoma. *Veterinary medicine international 2012,* 2012:761034.

De Jong, Y., Van Oosterwijk, J.G., Kruisselbrink, A.B., Briaire-de Bruijn, I.H., Agrogiannis, G., Baranski, Z., Cleven, A.H.G., Cleton-Jansen, A.M., Van De Water, B., Danen, E.H.J. and Bovée, J.V.M.G., 2016. Targeting survivin as a potential new treatment for chondrosarcoma of bone. *Oncogenesis*, 5:e222.

Dolman, M.E.M., den Hartog, I.J., Molenaar, J.J., Schellens, J.H., Beijnen, J.H. and Sparidans, R.W., 2014. Liquid chromatography-tandem mass spectrometric assay for the light sensitive survivin suppressant sepantronium bromide (YM155) in mouse plasma. *Journal of pharmaceutical and biomedical analysis*, 92:144-148.

Dresang, L.R., Guastafierro, A., Arora, R., Normolle, D., Chang, Y. and Moore, P.S., 2013. Response of merkel cell polyomavirus-positive merkel cell carcinoma xenografts to a survivin inhibitor. *PLoS One*, 8:e80543.

Evan, G.I. and Vousden, K.H., 2001. Proliferation, cell cycle and apoptosis in cancer. *Nature*, 411:342-348.

F. Barroga, E., Kadosawa, T., Okumura, M. and Fujinaga, T., 1999. Establishment and characterization of the growth and pulmonary metastasis of a highly lung metastasizing cell line from canine osteosarcoma in nude mice. *Journal of veterinary medical science*, 61:361-367.

Flory, A.B., Rassnick, K.M., Balkman, C.E., Kiselow, M.A., Autio, K., Beaulieu, B.B. and Lewis, L.D., 2008. Oral bioavailability of etoposide after administration of a single dose to tumor-bearing dogs. *American journal of veterinary research*, 69:1316-1322.

Fosslien, E., 2000. Biochemistry of cyclooxygenase (COX)-2 inhibitors and molecular pathology of COX-2 in neoplasia. *Critical reviews in clinical laboratory sciences*, 37:431-502.

Gallay-Lepoutre, J., Bélanger, M.C. and Nadeau, M.E., 2016. Prospective evaluation of Doppler echocardiography, tissue Doppler imaging and biomarkers measurement for the detection of doxorubicin-induced cardiotoxicity in dogs: A pilot study. *Research in veterinary science*, 105:153-159.

Gantchev, T.G. and Hunting, D.J., 1997. Enhancement of etoposide (VP-16) cytotoxicity by enzymatic and photodynamically induced oxidative stress. *Anti-cancer drugs*, 8:164-173.

Gerritsen‐van Schieveen, P., Royer, B. and "Therapeutic drug monitoring group of the French Society of Pharmacology and Therapeutics," 2011. Level of

evidence for therapeutic drug monitoring for etoposide after oral administration. *Fundamental & clinical pharmacology*, 25:277-282.

Grösch, S., Tegeder, I., Niederberger, E., Bräutigam, L. and Geisslinger, G., 2001. COX-2 independent induction of cell cycle arrest and apoptosis in colon cancer cells by the selective COX-2 inhibitor celecoxib. *The FASEB journal*, 15:2742-2744.

Guerriero, J.L., Ditsworth, D., Fan, Y., Zhao, F., Crawford, H.C. and Zong, W.X., 2008. Chemotherapy induces tumor clearance independent of apoptosis. *Cancer research*, 68:9595-9600.

Hanahan, D. and Weinberg, R.A., 2000. The hallmarks of cancer. *Cell*, 100:57-70.

Hande, K.R., 1998. Etoposide: four decades of development of a topoisomerase II inhibitor. *European journal of cancer*, 34:1514-1521.

Hanif, R., Pittas, A., Feng, Y., Koutsos, M.I., Qiao, L., Staiano-Coico, L., Shiff, S.I. and Rigas, B., 1996. Effects of nonsteroidal anti-inflammatory drugs on proliferation and on induction of apoptosis in colon cancer cells by a prostaglandin-independent pathway. *Biochemical pharmacology*, 52:237-245.

Harisi, R., Dudas, J., Nagy-Olah, J., Timar, F., Szendroi, M. and Jeney, A., 2007. Extracellular matrix induces doxorubicin-resistance in human osteosarcoma cells by suppression of p53 function. *Cancer biology & therapy*, 6:1251-1257.

Hawk, E.T., Viner, J.L., Dannenberg, A. and DuBois, R.N., 2002. COX-2 in cancer—a player that's defining the rules. *Journal of the National Cancer Institute*, 94:545-546.

Hayashi, N., Asano, K., Suzuki, H., Yamamoto, T., Tanigawa, N., Egawa, S. and Manome, Y., 2005. Adenoviral infection of survivin antisense sensitizes prostate cancer cells to etoposide *in vivo*. *The Prostate*, 65:10-19.

Hida, T., Kozaki, K.I., Muramatsu, H., Masuda, A., Shimizu, S., Mitsudomi, T., Sugiura, T., Ogawa, M. and Takahashi, T., 2000. Cyclooxygenase-2 inhibitor induces apoptosis and enhances cytotoxicity of various anticancer agents in non-small cell lung cancer cell lines. *Clinical Cancer Research*, 6:2006-2011.

Hohenhaus, A.E. and Matus, R.E., 1990. Etoposide (VP‑16). *Journal of Veterinary Internal Medicine*, 4:239-241.

Hong, S.H., Kadosawa, T., Mochizuki, M., Matsunaga, S., Nishimura, R. and Sasaki, N., 1998. Establishment and characterization of two cell lines derived from canine spontaneous osteosarcoma. *Journal of veterinary medical science* 60:757–760.

Jones, M.K., Wang, H., Peskar, B.M., Levin, E., Itani, R.M., Sarfeh, I.J. and Tarnawski, A.S., 1999. Inhibition of angiogenesis by nonsteroidal anti-

inflammatory drugs: insight into mechanisms and implications for cancer growth and ulcer healing. *Nature medicine*, 5:1418-1423.

Kadosawa, T., Nozaki, K., Sasaki, N. and Takeuchi, A., 1994. Establishment and characterization of a new cell line from a canine osteosarcoma. *Journal of Veterinary Medical Science*, 56:1167-1169.

Kang, H.K., Lee, E., Pyo, H. and Lim, S.J., 2005. Cyclooxygenase-independent down-regulation of multidrug resistance–associated protein-1 expression by celecoxib in human lung cancer cells. *Molecular cancer therapeutics*, 4:1358-1363.

Karpinich, N.O., Tafani, M., Rothman, R.J., Russo, M.A. and Farber, J.L., 2002. The course of etoposide-induced apoptosis from damage to DNA and p53 activation to mitochondrial release of cytochromec. *Journal of Biological Chemistry*, 277:16547-16552.

Kaufmann, S.H., Desnoyers, S., Ottaviano, Y., Davidson, N.E. and Poirier, G.G., 1993. Specific proteolytic cleavage of poly (ADP-ribose) polymerase: an early marker of chemotherapy-induced apoptosis. *Cancer research*, 53:3976-3985.

Kawasaki, H., Altieri, D.C., Lu, C.D., Toyoda, M., Tenjo, T. and Tanigawa, N., 1998. Inhibition of apoptosis by survivin predicts shorter survival rates in colorectal cancer. *Cancer research*, 58:5071-5074.

Khaled, M., Belaaloui, G., Jiang, Z.Z., Zhu, X. and Zhang, L.Y., 2016. Antitumor effect of Deoxypodophyllotoxin on human breast cancer xenograft transplanted in BALB/c nude mice model. *Journal of Infection and Chemotherapy*, 22:692-696.

Kita, A., Nakahara, T., Yamanaka, K., Nakano, K., Nakata, M., Mori, M., Kaneko, N., Koutoku, H., Izumisawa, N. and Sasamata, M., 2011. Antitumor effects of YM155, a novel survivin suppressant, against human aggressive non-Hodgkin lymphoma. *Leukemia research*, 35:787-792.

Kleffel, S., Lee, N., Lezcano, C., Wilson, B.J., Sobolewski, K., Saab, K.R., Mueller, H., Zhan, Q., Posch, C., Elco, C.P. and DoRosario, A., 2016. ABCB5-Targeted Chemoresistance Reversal Inhibits Merkel Cell Carcinoma Growth. *Journal of Investigative Dermatology*, 136:838-846.

Knapp, D.W., Glickman, N.W., Widmer, W.R., DeNicola, D.B., Adams, L.G., Kuczek, T., Bonney, P.L., DeGortari, A.E., Han, C. and Glickman, L.T., 2000. Cisplatin versus cisplatin combined with piroxicam in a canine model of human invasive urinary bladder cancer. *Cancer chemotherapy and pharmacology*, 46:221-226.

Knapp, D.W., Richardson, R.C., Bottoms, G.D., Teclaw, R. and Chan, T.C., 1992. Phase I trial of piroxicam in 62 dogs bearing naturally occurring tumors. *Cancer chemotherapy and pharmacology*, 29:214-218.

Knapp, D.W., Richardson, R.C., Chan, T.C., Bottoms, G.D., Widmer, W.R., DeNicola, D.B., Teclaw, R., Bonney, P.L. and Kuczek, T., 1994. Piroxicam therapy in 34 dogs with transitional cell carcinoma of the urinary bladder. *Journal of Veterinary Internal Medicine*, 8:273-278.

Kumar, B., Yadav, A., Lang, J.C., Cipolla, M.J., Schmitt, A.C., Arradaza, N., Teknos, T.N. and Kumar, P., 2012. YM155 reverses cisplatin resistance in head and neck cancer by decreasing cytoplasmic survivin levels. *Molecular cancer therapeutics*, 11:1988-1998.

Lana, S., U'ren, L., Plaza, S., Elmslie, R., Gustafson, D., Morley, P. and Dow, S., 2007. Continuous Low‐Dose Oral Chemotherapy for Adjuvant Therapy of Splenic Hemangiosarcoma in Dogs. Journal o*f Veterinary Internal Medicine*, 21:764-769.

Le Deley, M.C., Guinebretière, J.M., Gentet, J.C., Pacquement, H., Pichon, F., Marec-Bérard, P., Entz-Werlé, N., Schmitt, C., Brugières, L., Vanel, D. and Dupoüy, N., 2007. SFOP OS94: a randomised trial comparing preoperative high-dose methotrexate plus doxorubicin to high-dose methotrexate plus etoposide and ifosfamide in osteosarcoma patients. *European Journal of Cancer*, 43:752-761.

Lee, J.S., Takahashi, T., Hagiwara, A., Yoneyama, C., Itoh, M., Sasabe, T., Muranishi, S. and Tashima, S., 1995. Safety and efficacy of intraperitoneal injection of etoposide in oil suspension in mice with peritoneal carcinomatosis. *Cancer chemotherapy and pharmacology*, 36:211-216.

Lewis, K.D., Samlowski, W., Ward, J., Catlett, J., Cranmer, L., Kirkwood, J., Lawson, D., Whitman, E. and Gonzalez, R., 2011. A multi-center phase II evaluation of the small molecule survivin suppressor YM155 in patients with unresectable stage III or IV melanoma. *Investigational new drugs*, 29:161-166.

Li, F., Ambrosini, G., Chu, E.Y., Plescia, J., Tognin, S., Marchisio, P.C. and Altieri, D.C., 1998. Control of apoptosis and mitotic spindle checkpoint by survivin. *Nature*, 396:580-584.

Liang, H., Zhang, L., Xu, R. and Ju, X.L., 2013. Silencing of survivin using YM155 induces apoptosis and chemosensitization in neuroblastomas cells. *European Review for Medical and Pharmacological Sciences*, 17:2909-2915.

Lock, R.B., 1992. Inhibition of p34cdc2 kinase activation, p34cdc2 tyrosine dephosphorylation, and mitotic progression in Chinese hamster ovary cells exposed to etoposide. *Cancer Research*, 52:1817-1822.

MacEwen, E.G., Kurzman, I.D., Rosenthal, R.C., Smith, B.W., Manley, P.A., Roush, J.K., Howard, P.E., 1989. Therapy for osteosarcoma in dogs with intravenous injection of liposome-encapsulated muramyl tripeptide. *Journal of the National Cancer Institute*, 81:935-938.

Matsubara, K., Kubota, M., Adachi, S., Kuwakado, K., Hirota, H., Wakazono, Y., Akiyama, Y., Mikawa, H., 1994. Induction of apoptosis in childhood acute leukemia by chemotherapeutic agents: failure to detect evidence of apoptosis *in vivo*. *European Journal of Haematology*, 52:47-52.

Mauldin, G.E., Fox, P.R., Patnaik, A.K., Bond, B.R., Mooney, S.C., Matus, R.E., 1992. Doxorubicin-induced cardiotoxicosis: clinical features in 32 dogs. *Journal of Veterinary Internal Medicine*, 6:82-88.

Mauldin, G.N., Matus, R.E., Withrow, S.J., Patnaik, A.K., 1988. Canine osteosarcoma. *Journal of Veterinary Internal Medicine*, 2:177–180.

McDuffie, J.E., Sablad, M., Ma, J., Snook, S., 2010. Urinary parameters predictive of cisplatin-induced acute renal injury in dogs. *Cytokine*, 52:156–162.

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.

Meng, C., Lu, Z., Fang, M., Zhou, X., Dai, K., Zhang, S., Luo, J., Luo, Z., 2014. Effect of celecoxib combined with chemotherapy drug on malignant biological behaviors of gastric cancer. *International Journal of Clinical and Experimental Pathology*, 7:7622–7632.

Milas, L., Hunter, N.R., Kurdoglu, B., Mason, K.A., Meyn, R.E., Stephens, L.C. and Peters, L.J., 1995. Kinetics of mitotic arrest and apoptosis in murine mammary and ovarian tumors treated with taxol. *Cancer chemotherapy and pharmacology*, 35:297-303.

Minematsu, T., Felder, L., Oppeneer, T., Sakazume, M., Oikawa, K., Hashimoto, T., Usui, T., Kimamura, H., 2008. Liquid chromatography–electrospray tandem mass spectrometric assay suitable for quantitation of YM155, a novel small-molecule survivin suppressant, in dog plasma. *Biomedical Chromatography*, 22:763-769.

Minoda, M., Kawamoto, T., Ueha, T., Kamata, E., Morishita, M., Harada, R., Toda, M., Onishi, Y., Hara, H., Kurosaka, M., Akisue, T., 2015. Antitumor effect of YM155, a novel small-molecule survivin suppressant, via mitochondrial apoptosis in human MFH/UPS. *International Journal of Oncology*, 47:891-899.

Mir, R., Stanzani, E., Martinez-Soler, F., Villanueva, A., Vidal, A., Condom, E., Ponce, J., Gil, J., Tortosa, A., Giménez-Bonafé, P., 2014. YM155 sensitizes ovarian cancer cells to cisplatin inducing apoptosis and tumor regression. *Gynecologic Oncology*, 132:211-220.

Misdorp, W., Hart, A.A.M., 1979. Some prognostic and epidemiologic factors in canine osteosarcoma. *Journal of the National Cancer Institute*, 62:537–545. Abstract retrieved September 12, 2016 from Oxford Journals.

Mita, A.C., Mita, M.M., Nawrocki, S.T., Giles, F.J., 2008. Survivin: key regulator of mitosis and apoptosis and novel target for cancer therapeutics. *Clinical Cancer Research*, 14:5000–5005.

Mohammed, S.I., Bennett, P.F., Craig, B.A., Glickman, N.W., Mutsaers, A.J., Snyder, P.W., Widmer, W.R., DeGortari, A.E., Bonney, P.L., Knapp, D.W., 2002. Effects of the cyclooxygenase inhibitor, piroxicam, on tumor response, apoptosis, and angiogenesis in a canine model of human invasive urinary bladder cancer. *Cancer Research*, 62:356-358.

Mohammed, S.I., Knapp, D.W., Bostwick, D.G., Foster, R.S., Khan, K.N.M., Masferrer, J.L., Woerner, B.M., Snyder, P.W., Koki, A.T., 1999. Expression of cyclooxygenase-2 (COX-2) in human invasive transitional cell carcinoma (TCC) of the urinary bladder. *Cancer Research*, 59:5647-5650.

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.

Morris, J., Dobson, J., 2001. Small animal oncology. Blackwell Science, pp. 79-89.

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

Nakahara, T., Kita, A., Yamanaka, K., Mori, M., Amino, N., Takeuchi, M., Tominaga, F., Kinoyama, I., Matsuhisa, A., Kudou, M., Sasamata, M., 2011. Broad spectrum and potent antitumor activities of YM155, a novel small-molecule survivin suppressant, in a wide variety of human cancer cell lines and xenograft models. *Cancer Science*, 102:614-621.

Nam, C., Doi, K., Nakayama, H., 2010. Etoposide induces G2-M arrest and apoptosis in neural progenitor cells via DNA damage and an ATM-p53-related pathway. *Histology and Histopathology*, 25:485-493.

Novello, C., Pazzaglia, L., Conti, A., Quattrini, I., Pollino, S., Perego, P., Picci, P., Benassi, M.S., 2014. p53-dependent activation of microRNA-34a in response to etoposide-induced DNA damage in osteosarcoma cell lines not impaired by dominant negative p53. *PLoS One*, 9:e114757.

Oblak, M.L., Boston, S.E., Higginson, G., Patten, S.G., Monteith, G.J., Woods, J.P., 2012. The impact of pamidronate and chemotherapy on survival times in

dogs with appendicular primary bone tumors treated with palliative radiation therapy. *Veterinary Surgery*, 41:430-435.

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.

O'Connor, P.M., Ferris, D.K., White, G.A., Pines, J., Hunter, T., Longo, D.L., Kohn, K.W., 1992. Relationships between cdc2 kinase, DNA cross-linking, and cell cycle perturbations induced by nitrogen mustard. *Cell Growth and Differentiation*, 3:43-52.

O'kane, G.M., Cadoo, K.A., Walsh, E.M., Emerson, R., Dervan, P., O'Keane, C., Hurson, B., O'Toole, G., Dudeney, S., Kavanagh, *et al.*, 2015. Perioperative chemotherapy in the treatment of osteosarcoma: a 26-year single institution review. *Clinical Sarcoma Research*, 5:1.

Olie, R.A., Simões-Wüst, A.P., Baumann, B., Leech, S.H., Fabbro, D., Stahel, R.A., Zangemeister-Wittke, U., 2000. A novel antisense oligonucleotide targeting survivin expression induces apoptosis and sensitizes lung cancer cells to chemotherapy. *Cancer Research*, 60:2805-2809.

Ong, S.M., Yamamoto, H., Saeki, K., Tanaka, Y., Yoshitake, R., Nishimura, R., Nakagawa, T., 2017. Anti-neoplastic effects of topoisomerase inhibitors in canine mammary carcinoma, melanoma, and osteosarcoma cell lines. *Japanese Journal of Veterinary Research,* in press.

Orth, J.D., Kohler, R.H., Foijer, F., Sorger, P.K., Weissleder, R. and Mitchison, T.J., 2011. Analysis of mitosis and antimitotic drug responses in tumors by in vivo microscopy and single-cell pharmacodynamics. *Cancer Research*, 71:4608-4616.

Parker, L.L., Piwnica-Worms, H., 1992. Inactivation of the p34cdc2-cyclin B complex by the human WEE1 tyrosine kinase. *Science*, 257:1955-1957.

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.

Rankin, W.V., Henry, C.J., Turnquist, S.E., Turk, J.R., Beissenherz, M.E., Tyler, J.W., Rucker, E.B., Knapp, D.W., Rodriguez, C.O., Green, J.A., 2008. Identification of survivin, an inhibitor of apoptosis, in canine urinary bladder transitional cell carcinoma. *Veterinary and Comparative Oncology*, 6:141-150.

Rebhun, R.B., Lana, S.E., Ehrhart, E.J., Charles, J.B., Thamm, D.H., 2008. Comparative analysis of survivin expression in untreated and relapsed canine lymphoma. *Journal of Veterinary Internal Medicine*, 22:989-995.

Reddy, L.H., Adhikari, J.S., Dwarakanath, B.S.R., Sharma, R.K., Murthy, R.R., 2006. Tumoricidal effects of etoposide incorporated into solid lipid nanoparticles after intraperitoneal administration in Dalton's lymphoma bearing mice. *The AAPS Journal*, 8:E254–E262.

Reed, J.C., 1999. Dysregulation of apoptosis in cancer. *Journal of Clinical Oncology*, 17:2941-2953.

Rosa, J., Canovas, P., Islam, A., Altieri, D.C., Doxsey, S.J., 2006. Survivin modulates microtubule dynamics and nucleation throughout the cell cycle. Molecular Biology of the Cell, 17:1483-1493.

Rosenberger, J.A., Pablo, N.V., Crawford, P.C., 2007. Prevalence of and intrinsic risk factors for appendicular osteosarcoma in dogs: 179 cases (1996–2005). *Journal of the American Veterinary Medical Association*, 231:1076–1080.

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

Sarela, A.I., Macadam, R.C.A., Farmery, S.M., Markham, A.F., Guillou, P.J., 2000. Expression of the antiapoptosis gene, Survivin, predicts death from recurrent colorectal carcinoma. *Gut*, 46:645–650. doi:10.1136/gut.46.5.645

Satoh, T., Okamoto, I., Miyazaki, M., Morinaga, R., Tsuya, A., Hasegawa, Y., Terashima, M., Ueda, S., Fukuoka, M., Ariyoshi, Y., Saito, T., Masuda, N., Watanabe, H., Taguchi, T., Kakihara, T., Aoyama, Y., Hashimoto, Y., Nakagawa, K., 2009. Phase I study of YM155, a novel survivin suppressant, in patients with advanced solid tumors. *Clinical Cancer Research*, 15:3872-3880.

Schmidt, B.R., Glickman, N.W., DeNicola, D.B., Gortari, A.E.D., Knapp, D.W., 2001. Evaluation of piroxicam for the treatment of oral squamous cell carcinoma in dogs. *Journal of the American Veterinary Medical Association*, 218:1783-1786.

Schlüter, C., Duchrow, M., Wohlenberg, C., Becker, M.H.G., Key, G., Flad, H.D., Gerdes, J., 1993. The cell proliferation-associated antigen of antibody Ki-67: a very large, ubiquitous nuclear protein with numerous repeated elements, representing a new kind of cell cycle-maintaining proteins. *The Journal of Cell Biology*, 123:513–522.

Schwartz, C.L., Wexler, L.H., Krailo, M.D., Teot, L.A., Devidas, M., Steinherz, L.J., Goorin, A.M., Gebhardt, M.C., Healey, J.H., Sato, J.K., Meyers, P.A., Grier, H.E., Bernstein, M.L., Lipshultz, S.E., 2016. Intensified chemotherapy with dexrazoxane cardioprotection in newly diagnosed nonmetastatic osteosarcoma: A report from the Children's Oncology Group. *Pediatric Blood and Cancer*, 63:54-61.

Selvarajah, G.T., Kirpensteijn, J., 2010. Prognostic and predictive biomarkers of canine osteosarcoma. *The Veterinary Journal*, 185:28-35.

Sethi, T., Rintoul, R.C., Moore, S.M., MacKinnon, A.C., Salter, D., Choo, C., Chilvers, E.R., Dransfield, I., Donnelly, S.C., Strieter, R., Haslett, C., 1999. Extracellular matrix proteins protect small cell lung cancer cells against apoptosis: a mechanism for small cell lung cancer growth and drug resistance *in vivo*. *Nature Medicine*, 5:662-668.

Sharma, H., Sen, S., Muzio, L.L., Mariggiò, M.A., Singh, N., 2005. Antisense-mediated downregulation of anti-apoptotic proteins induces apoptosis and sensitizes head and neck squamous cell carcinoma cells to chemotherapy. *Cancer Biology & Therapy*, 4:720-727.

Shoeneman, J.K., Ehrhart, E.J., Eickhoff, J.C., Charles, J.B., Powers, B.E., Thamm, D.H., 2012. Expression and function of survivin in canine osteosarcoma. *Cancer Research*, 72:249-259.

Smeets, M.F.M.A., Mooren, E.H.M., Begg, A.C., 1994. The effect of radiation on G 2 blocks, cyclin B expression and cdc2 expression in human squamous carcinoma cell lines with different radiosensitivities. *Radiotherapy and Oncology*, 33:217-227.

Snyder, C.L., Saltzman, D.A., Ferrell, K.L., Thompson, R.C., Leonard, A.S., 1991. A new approach to the resection of pulmonary osteosarcoma metastases: results of aggressive metastatectomy. *Clnical Orthopaedics and Related Research*, 270:247-253.

Souza, C.H.D.M., Toledo-Piza, E., Amorin, R., Barboza, A., Tobias, K.M., 2009. Inflammatory mammary carcinoma in 12 dogs: Clinical features, cyclooxygenase-2 expression, and response to piroxicam treatment. *Canadian Veterinary Journal*, 50:506-510.

Souza, R.F., Shewmake, K., Beer, D.G., Cryer, B., Spechler, S.J., 2000. Selective inhibition of cyclooxygenase-2 suppresses growth and induces apoptosis in human esophageal adenocarcinoma cells. *Cancer Research*, 60:5767-5772

Spodnick, G., Berg, J., Rand, W., Schelling, S., Couto, G., Harvey, H., Henderson, R., MacEwen, G., Mauldin, N., McCaw, D., 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. Abstract retrieved February 6, 2016 from Europe PMC.

Spugnini, E.P., Cardillo, I., Verdina, A., Crispi, S., Saviozzi, S., Calogero, R., Nebbioso, A., Altucci, L., Cortese, G., Galati, R., Chien, J., Shridhar, V., Vincenzi, B., Citro, G., Cognetti, F., Sacchi, A., Baldi, A., 2006. Piroxicam and cisplatin in a mouse model of peritoneal mesothelioma. *Clinical Cancer Research*, 12:6133-6143.

Srinivas, R., Satterlee, A., Wang, Y., Zhang, Y., Wang, Y., Huang, L., 2015. Theranostic etoposide phosphate/indium nanoparticles for cancer therapy and imaging. *Nanoscale*, 7:18542-18551.

Stark, G.R., Taylor, W.R., 2004. Analyzing the G2/M checkpoint. Checkpoint Controls and Cancer. Volume 1: Reviews and Model Systems, 51-82.

Straw, R.C., Withrow, S.J., Richter, S.L., Powers, B.E., Klein, M.K., Postorino, N.C., LaRue, S.M., Ogilvie, G.K., Vail, D.M., Morrison, W.B., McGee, M., Dickinson, K., 1991. Amputation and cisplatin for treatment of canine osteosarcoma. *Journal of Veterinary Internal Medicine*, 5:205-210.

Su, B.-H., Shieh, G.-S., Tseng, Y.-L., Shiau, A.-L., Wu, C.-L., 2015. Etoposide enhances antitumor efficacy of MDR1-driven oncolytic adenovirus through autoupregulation of the MDR1 promoter activity. *Oncotarget*, 6:38308–38326.

Symmans, W.F., Volm, M.D., Shapiro, R.L., Perkins, A.B., Kim, A.Y., Demaria, S., Yee, H.T., McMullen, H., Oratz, R., Klein, P. and Formenti, S.C., 2000. Paclitaxel-induced apoptosis and mitotic arrest assessed by serial fine-needle aspiration: implications for early prediction of breast cancer response to neoadjuvant treatment. Clinical Cancer Research, 6:4610-4617.

Szepeshazi, K., Lapis, K. and Schally, A.V., 1991. Effect of combination treatment with analogs of luteinizing hormone-releasing hormone (LH-RH) or somatostatin and 5-fluorouracil on pancreatic cancers in hamster. *International Journal of Cancer,* 49:260-266.

Szewczyk, M., Lechowski, R., Zabielska, K., 2014. What do we know about canine osteosarcoma treatment? *Veterinary Research Communications*, 39:61-67.

Taromi, S., Kayser, G., Catusse, J., von Elverfeldt, D., Reichardt, W., Braun, F., Weber, W.A., Zeiser, R., 2016. CXCR4 antagonists suppress small cell lung cancer progression. *Oncotarget*, 7:85185-85195.

Thompson, C.B., 1995. Apoptosis in the pathogenesis and treatment of disease. *Science*, 267:1456–1462.

Tolcher, A.W., Mita, A., Lewis, L.D., Garrett, C.R., Till, E., Daud, A.I., Patnaik, A., Papadopoulos, K., Takimoto, C., Bartels, P., Keating, A., Antonia, S., 2008. Phase I and pharmacokinetic study of YM155, a small-molecule inhibitor of survivin. *Journal of Clinical Oncology*, 26:5198-5203.

Uetsuka, H., Haisa, M., Kimura, M., Gunduz, M., Kaneda, Y., Ohkawa, T., Takaoka, M., Murata, T., Nobuhisa, T., Yamatsuji, T., Matsuoka, J., Tanaka, N., Naomoto, Y., 2003. Inhibition of inducible NF-κB activity reduces chemoresistance to 5-fluorouracil in human stomach cancer cell line. *Experimental Cell Research*, 289:27-35.

Vail, D.M., Kurzman, I.D., Glawe, P.C., O'Brien, M.G., Chun, R., Garrett, L.D., Obradovich, J.E., Fred, 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 Wijngaarden, J., van Beek, E., van Rossum, G., van der Bent, C., Hoekman, K., van der Pluijm, G., van der Pol, M.A., Broxterman, H.J., van Hinsbergh, V.W., Löwik, C.W., 2007. Celecoxib enhances doxorubicin-induced cytotoxicity in MDA-MB231 cells by NF-κB-mediated increase of intracellular doxorubicin accumulation. *European Journal of Cancer*, 43:433-442.

Wang, Y., Wang, C., Jiang, C., Zeng, H., He, X., 2015. Novel mechanism of harmaline on inducing G2/M cell cycle arrest and apoptosis by up-regulating Fas/FasL in SGC-7901 cells. *Scientific Reports*, 5.

Wang, Y.F., Zhang, W., He, K.F., Liu, B., Zhang, L., Zhang, W.F., Kulkarni, A.B., Zhao, Y.F., Sun, Z.J., 2014. Induction of autophagy-dependent cell death by the survivin suppressant YM155 in salivary adenoid cystic carcinoma. *Apoptosis*, 19:748-758.

Whelan, J.S., Bielack, S.S., Marina, N., Smeland, S., Jovic, G., Hook, J.M., Krailo, M., Anninga, J., Butterfass-Bahloul, T., Böhling, T., Calaminus, G., Capra, M., Deffenbaugh, C., Dhooge, C., Eriksson, M., Flanagan, A.M., Gelderblom, H., Goorin, A., Gorlick, R., Gosheger, G., Grimer, R.J., Hall, K.S., Helmke, K., Hogendoorn, P.C.W., Jundt, G., Kager, L., Kuehne, T., Lau, C.C., Letson, G.D., Meyer, J., Meyers, P.A., Morris, C., Mottl, H., Nadel, H., Nagarajan, R., Randall, R.L., Schomberg, P., Schwarz, R., Teot, L.A., Sydes, M.R., Bernstein, M. 2015. EURAMOS-1, an international randomised study for osteosarcoma: results from pre-randomisation treatment. *Annals of Oncology*, 26:407-414.

Wyllie, A.H., 1986. What is apoptosis?. *Histopathology*, 10:995-998.

Yamanaka, K., Nakahara, T., Yamauchi, T., Kita, A., Takeuchi, M., Kiyonaga, F., Kaneko, N., Sasamata, M., 2011. Antitumor activity of YM155, a selective small-molecule survivin suppressant, alone and in combination with docetaxel in human malignant melanoma models. *Clinical Cancer Research*, 17:5423-5431.

Yamazaki, H., Takagi, S., Hosoya, K., Okumura, M., 2015. Survivin suppressor (YM155) enhances chemotherapeutic efficacy against canine histiocytic sarcoma in murine transplantation models. *Research in Veterinary Science*, 99,:137-144.

Yan, Y.X., Li, W.Z., Huang, Y.Q., Liao, W.X., 2012. The COX-2 inhibitor Celecoxib enhances the sensitivity of KB/VCR oral cancer cell lines to vincristine by down-regulating P-glycoprotein expression and function. *Prostaglandins and other Lipid Mediators*, 97:29-35

Zaffaroni, N., Daidone, M.G., 2002. Survivin expression and resistance to anticancer treatments: perspectives for new therapeutic interventions. *Drug Resistance Updates*, 5:65-72.

Zaffaroni, N., Pennati, M., Colella, G., Perego, P., Supino, R., Gatti, L., Pilotti, S., Zunino, F., Daidone, M.G., 2002. Expression of the anti-apoptotic gene survivin correlates with taxol resistance in human ovarian cancer. *Cellular and Molecular Life Sciences CMLS*, 59:1406-1412.

Zandvliet, M.M.J.M., Teske, E., Chapuis, T., Fink-Gremmels, J., Schrickx, J.A., 2013. Masitinib reverses doxorubicin resistance in canine lymphoid cells by inhibiting the function of P-glycoprotein. *Journal of Veterinary Pharmacology and Therapeutics,* 36:583-587.

Zhang, M., Mukherjee, N., Bermudez, R.S., Latham, D.E., Delaney, M.A., Zietman, A.L., Shipley, W.U., Chakravarti, A., 2005. Adenovirus-mediated inhibition of survivin expression sensitizes human prostate cancer cells to paclitaxel *in vitro* and *in vivo*. *The Prostate,* 64:293-302.

Zhang, X.Q., Sun, X.E., Liu, W.D., Feng, Y.G., Zhang, H.M., Shi, L.H., Sun, X.N., Li, Y.Q., Gao, Z.X., 2015. Synergic effect between 5-fluorouracil and celecoxib on hypoxic gastric cancer cells. *Molecular Medicine Reports*, 11:1160-1166.

Zhang, Z., Vuori, K., Reed, J.C., Ruoslahti, E., 1995. The α5β1 integrin supports survival of cells on fibronectin and up-regulates Bcl-2 expression. *Proceedings of the National Academy of Sciences USA*, 92:6161-6165.

Zhang, Z., Zhang, Y., Lv, J., Wang, J., 2015. The survivin suppressant YM155 reverses doxorubicin resistance in osteosarcoma. *International Journal of Clinical and Experimental Medicine*, 8:18032-18040.

Zimmermann, G.R., Lehar, J., Keith, C.T., 2007. Multi-target therapeutics: when the whole is greater than the sum of the parts. *Drug Discovery Today*, 12:34-42.

Zrieki, A., Farinotti, R., Buyse, M., 2008. Cyclooxygenase inhibitors down regulate P-glycoprotein in human colorectal Caco-2 cell line. *Pharmaceutical Research*, 25:1991-2001.