day 0 ($P = 0.019$) and day 42 ($P < 0.001$). There was no significant difference in respiratory burst between day 0 and 42 in the lymphoma group or in the response to PMA in normal and lymphoma dogs.

This data suggests that dogs with lymphoma have normal phagocytic function but decreased respiratory burst, and that respiratory burst function is not regained in remission. This implies that dogs with lymphoma have innate immune dysfunction compared to healthy dogs.

(VCS Award Winner)

O-3

CK2 INHIBITION IN FELINE CANCER CELL LINES USING SYNTHETIC OLIGONUCLEOTIDES. C Cannon[1], J Trembley[2,3], J Modiano[1,4], O Cespedes Gomez[3], B Kren[3,5], G Unger[2,3,6], K Ahmed[2,3,4]. [1]University of Minnesota College of Veterinary Medicine, St Paul MN, [2]University of Minnesota Department of Laboratory Medicine and Pathology, Minneapolis MN, [3]Minneapolis Veterans Administration Health Care System, Minneapolis MN, [4]University of Minnesota Masonic Cancer Research Center, Minneapolis MN, [5]University of Minnesota Department of Medicine, Minneapolis MN, [6]GeneSegues Inc, Chaska, MN.

We examined the effects of CK2 downregulation on feline cancer cell lines. CK2 is a serine/threonine protein kinase complex that is essential for cell growth and survival and is also a potent suppressor of apoptosis. Increased expression has been found in most human cancers, making it an attractive therapeutic target. We hypothesized that CK2 downregulation would result in impaired growth and survival in feline cancer cells.

We verified the target sequence of feline CK2α and CK2α' by DNA sequence analysis of polymerase chain reaction products from peripheral blood cells from two healthy cats and from the feline SCCF-1 and K12 laryngeal squamous and mammary carcinoma cell lines. CK2 expression in feline oral squamous cell carcinomas (FOSCC) was assessed using immunofluorescence. SCCF-1 and K12 cell lines were treated with anti-CK2 oligonucleotides (OGNs) and CK2 expression and growth inhibition in treated cells were determined using immunoblotting and thymidine incorporation, respectively.

FOSCC samples showed overexpression of CK2 in tumour cells compared to adjacent normal tissue. OGNs directed against the CK2α and CK2α' subunits caused a time dependent decrease in CK2 protein expression and inhibited growth.

Our results show that CK2 expression can be inhibited in feline tumour cells by targeting CK2α and CK2α' subunits using OGNs and that reduction in CK2 is associated with growth inhibition. A nanocapsule delivery system has been shown to be safe in laboratory animals, with effective tumor targeting, paving the way to test safety and efficacy of this approach in pre-clinical studies of cats with spontaneously occurring tumors.

O-4

THE INFLUENCE OF SURVIVIN INHIBITION ON INHIBITORY ACTIVITY AGAINST CANINE HISTIOCYTIC SARCOMA CELL LINES. H Yamazaki, S Takagi, Y Hoshino, K Hosoya, M Okumura. Department of Veterinary Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Hokkaido, Japan.

Survivin is a member of the inhibitor of apoptosis (IAP) family, which inhibits apoptosis and cell proliferation in many types of human cancer cells, and is expected to be a novel agent for human cancer therapy. Canine histiocytic sarcoma (HS) requires highly effective medical therapy because of its aggressive local infiltration and very high metastatic rate. The aim of this study was to evaluate the influence of **cell growth rate, chemosensitivity, and** phagocytic activity by survivin inhibition in canine HS cell lines.

Canine survivin siRNA and YM155 (a small-molecule suppressor of human survivin) were used as survivin inhibitors. Seven HS cell lines, including 3 chemoresistant HS cell lines, and canine fibroblasts as the normal cell control were used in this study. Annexin V staining for apoptosis evaluation and MTT assay for cell growth rate and chemosensitivity were performed.

After transfection with canine survivin siRNA, induction of apoptosis, decrease in cell growth rate, and enhancement of chemosensitivity were observed in all HS cells. After administration of YM155, these changes were observed in 3 of 7 HS cell lines, and the other 4 HS cell lines showed only slight down-regulation by YM155. In addition, fibroblasts were influenced only to a negligible extent by survivin inhibition.

In conclusion, survivin was related to the inhibitory activities against canine HS. Thus, survivin-targeted therapy might constitute a potentially useful novel therapeutic approach for canine HS.

O-5

SIGNIFICANCE OF A DIAGNOSTIC APPROACH BY USING SURFACE ANTIGENMRNA EXPRESSION ANALYSIS IN 8 DOGS WITH HISTIOCYTIC SARCOMA. S Takagi, H Yamazaki, K Hosoya, Y Hoshino, M Okumura. Department of Veterinary Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Hokkaido, Japan.

Canine histiocytic sarcomas (HS) have aggressive local infiltration and very high metastatic rates. The cellular morphology of canine HS shows highly undifferentiated cells with a mixed pattern of round-cell and mesenchymal appearance. However, these tumors are often necrotic and inflammatory. These features can sometimes be misleading, making a definitive diagnosis difficult. Immunohistochemical staining using cell surface antigens (CD11b, CD11c, CD86, and MHC class II antigens) is extremely helpful for the final diagnosis of this tumor; however, this technique requires special antigens and frozen samples.

Real-time (RT)-PCR is a method that can quantitate mRNA levels. We established an RT-PCR method to analyze the abovementioned surface antigens. The aim of this study was to apply this technique to fine-needle aspirated (FNA) samples and evaluate its accuracy.

Eight dogs with suspected histiocytic sarcoma were included in the present study. FNA samples were collected from sarcoma masses. Normal tissues of healthy dogs from sites that corresponded to tumor origin sites in affected dogs were also analyzed for mRNA levels of each antigen. Tissues obtained from surgical biopsy or autopsy were used for histopathological diagnosis, including immnunohistochemical staining.

Biopsied samples could not lead to a definitive diagnosis, while RT-PCR showed more than 200 times mRNA levels of each antigen compared with normal tissues. In some dogs, the final diagnosis could not be confirmed by the histopathological method until postmortem examination.

These results indicated that cell surface antigen analysis by using RT-PCR has prospects of clinical application.

O-6

ELECTRO-GENE-TRANSFER (EGT) AS A NEW TOOL FOR CANCER IMMUNOTHERAPY IN ANIMALS. Joseph A Impellizeri[1], Alessandra Gavazza[2], George Lubas[2], David Jemiolo[3], Gennaro Ciliberto[4], Luigi Aurisicchio[5]. [1]Veterinary Specialty Center of the Hudson Valley, Wappinger Falls, NY, [2]University of Pisa, Dept. of Veterinary Clinics, Pisa, Italy, [3]Vassar College, Poughkeepsie, NY, [4]National Cancer Institute "G. Pascale", Naples, Italy, [5]Takis, Rome, Italy.

The development of vaccines against cancer in human oncology is gaining increasing importance as a therapeutic approach which can complement standard chemotherapy and/or targeted

therapies to achieve increased survival and improved quality of life for patients. In the specialty of veterinary oncology, there is an unmet need for additional therapeutic interventions such as immunotherapy, due to increased demand of owners seeking advanced options for cancer treatment for their pet. The use of high pressure, needleless devices as an enhancing tool for plasmid DNA delivery led to recent approval by USDA of Oncept™, a therapeutic cancer vaccine directed against tyrosinase for the therapy of melanoma in dogs.

An alternative approach to improve plasmid DNA delivery is Electro-Gene-Transfer (EGT). In vivo electro-gene-transfer of plasmid DNA (DNA-EGT) is a safe methodology resulting in greater DNA cell uptake, enhanced protein expression and concomitant increase in longer term immune responses against the target antigen in a variety of species. The approach uses brief electrical pulses which create transient "pores" in the cell membranes that allow large molecules such as DNA or RNA to enter the cell cytoplasm. We have set up an optimal vaccination protocol in companion animals by means of a device, currently utilized and approved in Europe for Electrochemotherapy applications and adapted to Electro-Gene-Transfer.

We show that DNA-EGT can induce strong immune response against dog telomerase or dog HER2/neu targets especially when combined with Adenoviral (Ad) vectors. The vaccine resulted in significant therapeutic effects in dogs affected by B-cell malignant lymphosarcoma (LSA). The evaluation for other tumor types, such as mesothelioma, hemangiosarcoma, melanoma and renal cancer is in progress. No adverse effects have been observed in any canine or feline patient that could be attributed to immunotherapy.

We have established an optimized procedure for DNA-EGT. The technology is simple, safe and most importantly the vaccine prolongs the survival of canine patients when combined with Standard of Care. Thus, DNA-EGT holds promise as an adjunct therapy for the treatment of cancer in dogs and cats.

O-7

RELATIONSHIP BETWEEN SYSTEMIC INFLAMMATORY RESPONSE (CRP AND HP) AND LOCAL INFLAMMATORY RESPONSE (COX-2 EXPRESSION) IN CANINE MAMMARY TUMOURS. M Planellas, J Martínez, R Cuenca, J Castro, J Pastor. Faculty of Veterinary Medicine. Universitat Autònoma de Barcelona, Barcelona, Spain.

Mammary tumours (MT) are among the most commonly encountered neoplasm in dogs. Forty one to fifty-three per cent of all mammary tumours in dogs are malignant, with carcinoma being the most common malignant type. Cyclooxygenase-2 (COX-2) expression in human tumours can be induced by growth factors, cytokines, oncogenes and other factors. Induction of COX-2 has been implicated in various cancers. Recently, systemic inflammatory response, as evidenced by elevated circulating concentrations of acute phase proteins, has been shown to be independently associated with poorer survival in human patients with advanced cancer. Elevated C-reactive protein identifies tumours capable of producing significant amounts of proinflammatory cytokines, in particular interleukin-6, and therefore with the potential for more rapid growth of tumour cells. The purpose of this work was to study the relationship between the systemic inflammatory response (C-reactive protein, CRP and Haptoglobin, Hp) and local inflammatory response (COX-2 expression) in different canine mammary tumors.

Serum concentrations of CRP and Hp were determined in serum samples from healthy dogs (n = 20) and dogs with mammary tumors (n = 41), prior to surgery. Forty one mammary tumours were submitted for histopathological and immunohistochemical study. Mammary tumour type was determined by histopathologic examination according WHO Guidelines. From all studied samples, 32 were malignant MT and 9 were benign MT. Simple or complex adenomas (n = 3), mixed benign tumours (n = 6), carcinomas (n = 24), fibrosarcoma (n = 1) and mixed malignant tumour (n = 7) were studied by immunohistochemistry for COX-2 expression. A gradation score according the percentage of cells expressing COX-2 and the intensity was applied.

All the adenomas and 5 out of 6 (83.3%) mixed benign tumours were COX-2 positive. Immunostaining of malignant tumours revealed that 4 of 7 (57.14%) mixed malignant tumors and 12 of 24 (50%) carcinomas were COX-2 positive. CRP levels ranged from 0.05-70.6 mg/L, with a mean value of 1.7 mg/L. Hp values ranged from 0.11-128.9 g/L, with mean value of 9.69 g/L. No significant differences were observed between benign and malignant MT according COX-2 expression and intensity. No significant correlation has been found between CRP and Hp levels and COX-2 expression or intensity in MT. According our results, there is no association between systemic and local inflammatory response in canine mammary tumors.

O-8

THE EFFECTS OF SULFORAPHANE ON NEOPLASTIC CELL PROLIFERATION AND DEATH. V Rizzo, J Wakshlag, J Chandler. Cornell University College of Veterinary Medicine, Ithaca, NY.

Recent evidence in human cancer patients and rodent models suggests that the neutraceutical sulforaphane (typically found in raw cruciferous vegetables) may have utility in prevention of cisplatin induced renal toxicity, however there is little examination on its effects on cancer cells with or without conjunctive chemotherapeutics. To examine the potential safety and effects on canine cancer cells three canine cell lines were treated with a serial dilution of sulforaphane (0-50 uM) for 48 hours and 6 day growth curve assays were performed using MTT assays. Apoptosis was examined using annexin V staining, or caspase 3 immunoblotting. Further viability assays were performed to examine sulforaphane treatment in conjunction with either palladia (mast cells) or doxorubicin (osteosarcoma). Statistical analysis was performed using analysis of variance with Tukey's post hoc analysis or Student's T test to determine significance. Results suggest that all cell lines have reduced cell viability in both the 48 hour MTT proliferation and the growth curve analysis at between 2 and 3 uM of sulforaphane ($p < 0.05$) with apoptosis occurring in two of the three cell lines (mast cells and osteosarcoma cells) at concentrations between 10-20 uM. Sulforaphane did not promote cell survival nor did it promote further cell death in the face of other chemotherapeutic insults in mast or osteosarcoma cells. Further mechanisms for reduced cell viability are being examined, however sulforaphane does not confer a survival advantage for these cell lines making it a potential neutraceutical to investigate for chemotherapy-induced renal cytotoxicity.

O-9

TARGETING LYMPHOID PROGENITOR CELLS IN CANINE B-CELL LYMPHOMA USING NEOADJUVANT ABCB1 TRANSPORTER INHIBITION AND DOXORUBICIN CHEMOTHERAPY. D Ito[1], JF Modiano[1], MO Childress[2], NJ Mason[3], JF Leary[2], TD O'Brien[1], MS Henson[1], A Borgatti[1], E Krick[3], KM Stuebner[1], A Winter[1], JC Stewart[2], SA Lahrman[2], JL Meyers[2], S Ruetz[4]. [1]University of Minnesota, Minneapolis/St. Paul, MN, [2]Purdue University, West Lafayette, IN, [3]University of Pennsylvania, Philadelphia, PA, [4]Novartis Inc. Basel, Switzerland.

We previously described a population of lymphoid progenitor cells (LPCs) in canine lymphoma that persist in the xenotransplantation setting. Our hypothesis was that LPCs behave like tumor-initiating cells and that PSC-833, a selective inhibitor of the ATP binding cassette B1 transporter (ABCB1, a.k.a., p-glycoprotein/multidrug resistance protein-1) would sensitize them to doxorubicin and improve remission. Dogs with therapy-naïve diffuse large B-cell lymphoma were eligible to participate. Twenty dogs were enrolled into a double-blinded, placebo controlled, multi-institutional study. The experimental and control groups received oral PSC-833 (7.5 mg/kg) or placebo twice daily for 5 days, respectively, followed by 5 doses of doxorubicin 21 days