SYNOPSIS OF PRESENTATIONS AT THE CANINE CANCER CONFERENCE ENTITLED:

GENES, DOGS AND CANCER; EMERGING CONCEPTS IN MOLECULAR DIAGNOSIS AND THERAPY

Hosted by AMC Cancer Research Center, May 21-22, 2001
and
Sponsored by the AKC Canine Health Foundation and the Heska Corporation

Keynote Speaker: Dr. Lawrence A. Loeb, University of Washington, Seattle;
Mutations and cancer: Extensive experiments indicate a causal association between mutations and cancer, both in animals and in humans. Mutations arise when the amount of DNA damage exceeds the capacity of cellular mechanism for DNA repair. Unrepaired DNA lesions that miscode during DNA replication lead to mutations, some of which involve key genes that are responsible for malignant transformation. DNA damage can result from environmental exposure to chemicals as well as from the production of reactive molecules in cells by normal metabolic processes. In humans, it takes some 20 years from the time of carcinogenic exposure to the clinical detection of a tumor. Shorter-lived animals, such as the dog, could provide a system to monitor the appearance of malignancies as a function of mutation accumulation.

Mutations might not only initiate the malignant process, but also might be required for malignant progression. Because of the high frequency of chromosomal abnormalities and mutations, Dr. Loeb offered the hypothesis that cancer is manifested by a mutator phenotype. The mutator phenotype hypothesis proposes that mutations in genes required to maintain genomic instability are early events in the evolution of a tumor. Another hypothesis is that cancers arise by repetitive waves of clonal selection. Recent studies suggest that both a mutator phenotype and clonal selection are operative during tumor progression and moreover they are interdependent. With successive waves of clonal selection, one simultaneously selects for mutators within a tumor cell population.

Understanding mechanisms that generate a mutator phenotype has important implications for cancer prevention. For many tumors, a delay in the rate of accumulation of mutations by a factor as low as two could drastically reduce death rates from these tumors by extending the expected age of death due to cancer beyond the usual age of death due to other causes. As an example, by halving the mutation rate of human lung cancer cells, persons with cancer onset at 35 years of age would, on the average, die at age 75 instead of age 55. Likewise, men with prostate cancer with onset at 50 years of age would die at age 120 instead of age 85. By the time people would reach 75 or 120 years of age, they are likely to have died due to other cause, thus reducing the cancer death rates greatly. Because of the shorter life span of dogs as compared to humans, any reduction in the rate of mutation accumulation, they would have an even greater reduction in cancer death risk.

Current information about cancer development indicates that cancer cells grow in a field of cells with mutations and that these mutations accumulate over time. For this reason, the speaker cautioned that manipulation of a suppressor gene may not have a sustainable positive effect. Furthermore, gene therapy must reach most tumor cells and the field of potentially tumorous cells in which tumor cells grow in order to be effective. For these reasons, the speaker believes that the new cancer interventions under development will be most effective as early preventive interventions when only a few tumor cells exist.

Dr. Terri M. King, University of Texas-Houston, Health Science Center Medical School; Epidemiology of cancer: the genetic link: Dr. King estimated that 45% of elderly dogs die of cancer. More dogs appear to be living into older age when cancer risk is higher. The increase in longevitiy appears to be due to a number of reasons, including better care provided by owners, better nutrition, and more dogs receiving better veterinary services. Lymphoma is the most common hematopoietic canine tumor. Mammary cancer is the most common malignancy in female dogs.

There are several reasons for the complimentary nature of cancer research in dogs and humans. Tumor progression is similar in dogs and man. There are more physiological and genetic similarities between the dog and humans, than between the mouse and humans. They also share a common environment, thus common exposure to environmental carcinogens. Dogs have a shorter life span and experience faster tumor progression. Therefore the evaluation of novel treatment modalities requires less time in dogs as compared to humans. The inbreeding practiced in dog breeding helps the researcher to map rare genes.

Dr. King is conducting a research project on malignant histiocytomas (MH) in the Flat-Coated Retriever. The estimated incidence rate for all cancer in FCR is approximately 3 times higher than in the general canine population. Based on the age-specific numbers of all cancer cases diagnosed in FCR, they appear to have a bimodal age distribution: 3.5 and 7.5 years respectively. However, age specific population data for FCR is lacking. Therefore, it is not know if this distribution reflects higher numbers of dogs in these categories or a true increase in risk of developing ML in those ages. A risk factor study found no association between MH and coat color, age at neutering, medical treatment (including heartworm) of the dog, flea exposure, nor numbers of litters or puppies. Significant protective effects were observed for competition in hunting activities and lean body condition. The reason or reasons for these associations are not known. The researcher now has constructed a pedigree of over 8,000 FCR for more in-depth study of environmental risk factors, inbreeding, segregation analysis, and cytogenetic studies. The researcher emphasized the need for canine cancer statistics that will permit disequilibrium, Monte-Carlo, variance component model and other epidemiologic and genetic research analyses.

Dr. Elaine A. Ostrander, Fred Hutchinson Cancer Research Center, Seattle; Development and application of tools and approaches for genetic mapping of cancer susceptibility genes in dogs: Dr. Ostrander is concentrating on 3 approaches: High risk families, heritable risk factors and clues from evolution. Dogs are desirable disease research subjects because their breed structure reduces locus (gene location) heterogeneity. For example, if there are 10 cancer susceptibility genes for mammary cancer, one might expect to find only 2 or 3 susceptibility genes within a specific breed.

She reported that a manuscript describing a 1,800 marker canine genome map is in press and that a 3,000 marker map will be completed soon. The Minimal Screening Set of 172 microsatelite markers (MSS1) was described and ways for individual researchers to use this resource in genome-wide screening were provided.

Recent analysis of linkage disequalibrium in various breeds of dogs was described. Breeds that developed from few founders or narrow bottlenecks, resulting in limited locus heterogeneity, are advantageous for genetic research because relatively few pedigrees would be required to perform linkage analysis. For example, as few as 50 dogs may be needed to find a disease allele. The following future areas of research and research support were encouraged: map building, genetic mapping/locus heterogeneity, phenotyping, statistical issues, cancer epidemiology including cohort studies, access to data, tumor banks, and dog community resources.

Dr. Matthew Breen, Animal Health Trust, Newmarket, Suffolk, England; Canine molecular cytogenetics-development of resources and their application to studies of canine cancer: Most tumor cells have grossly abnormal karyotypes (chromosome composition) with structural and/or numerical aberrations (alterations/departures from normal). While many of these aberrations may be random, reflecting the general degree of genome instability in tumors, some are tumor-specific, indicating regions of the genome that hold important clues to the mechanisms of carcinogenesis and directing research into improving diagnosis, prognosis and treatment of certain cancers.

A fundamental need in all genetic research in the dogs is a standardized canine karyotype. This researcher has sorted and identified the 38 pairs of somatic chromosomes, plus the X and Y chromosomes. His nomenclature for these chromosomes has be accepted and used in most of the recent canine genetics research reports so as to standardize scientific protocols and improve communications.

Recent molecular cytogenetic evaluations of tumors using fluorescence in situ hybridization (FISH) techniques has revolutionized the way in which the genomic status of tumors is studied. Building upon the standardized nomenclature, direct analysis of tumor derived chromosomes with FISH using chromosome-specific probes, is a powerful technique that allows a rapid assessment of the gross numerical and structural characteristics of a cell. Comparative genomic hybridization (CGH) analysis also provides a detailed and accurate analysis of imbalanced chromosomal material within a single FISH reaction. A combination of both FISH and CGH is a strategy that will maximize the opportunities for the identification of chromosome aberrations.

Dr. Anne C. Avery, Colorado State University, Fort Collins: PCR for antigen receptor rearrangement (PARR): high sensitivity detection of lymphoid malignancies: The detection of canine canine lymphocytic malignancies has relied on the cytologic assessment of lymphocytes or the histologic examination of biopsy material. The diagnosis is often straightforward in advanced cases, but there are situations that present a diagnostic challenge. For example early stage lymphomas that might be difficult to distinguish from lymphoid hyperplasia and biopsies not fully representing the lesion. Because of these limitations, the researcher and associates have developed a more objective assay for the detection of lymphoma. The assay (PCR for antigen receptor rearrangement, or PARR) detects clonally expanded populations of lymphocytes by the size of genes encoding their antigen receptors. Any sample from which DNA can be extracted has been used in this test (blood, cavity fluid, cerebrospinal fluid, bone marrow, aspirated, and biopsies).

Using histologically/cytologically and clinically confirmed lymphomas as the gold standard for detection of lymphoma (n=199), or animals with other disease and 1 year of clinical follow-up (n=55) as the lymphoma negative group, they find that the PARR assay has 87% sensitivity and 99% specificity. The assay allows simultaneous phenotyping of most tumors. They have found that most multicentric lymphomas are B cell in origin, but that a high percentage of occult lymphomas are T cell in origin. The most important part of their study is that lymphoma/leukemia can underlie a number of conditions and escape cytologic or histologic detection. Also, lymphoid neoplasia is a heterogeneous set of diseases that often go undiagnoses using traditional methods. The most common of these occult lymphoid neoplasms appears to be a T cell neoplasm associated with peripheral cytopenias. Experience with their testing program underscores the importance of developing similar assay for other types of neoplastic processes.

Dr. Cheryl A. London, University of California, Davis; c-kit mutations and mast cell cancer: Mast cell tumors (MCT) are the most frequently diagnose malignant tumor of the dog. Their natural history is difficult to predict as they can behave in an extremely aggressive or benign manner. The proto-oncogene c-kit is known to play a critical role in the development and function of mast cells. Mutations in c-kit leading to constitutive activation of Kit have been identified in several malignant mast cell lines, as well as from some human patients with various forms of mastocytosis. Dr. London and associates demonstrated the presence of novel mutations in c-kit in spontaneous canine MCTs. Extensive analysis of archived MCT specimens at the Veterinary Medical Teaching Hospital at UC Davis revealed that this mutation in c-kit was present in those tumors more likely to behave in a malignant manner, with an incidence of over 40% in Grade III MCTs. They investigated Kit signaling and found that a particular kinase was constitutively associated with the mutant Kit.

They are currently in the process of developing mouse models for Kit dysfunction in which the expression of various forms of the active Kit is controlled by a promoter. The integration of detailed investigations of Kit dysregulation in the mouse and comprehensive studies of a spontaneous model of c-kit mutations in the dog are expected to clarify the biological and biochemical consequences of c-kit mutations and likely result in significant new information with broad applicability to human cancers as well as to the development and implementation of novel therapeutic strategies. The recent announcement of a Norartis pharmaceutical product, Gleevec, inhibiting Kit signaling resulted in over 50% remissions of human cancers, is very encouraging that similar c-kit research in the dog will be fruitful.

Dr. Jamie F. Modiano, AMC Cancer Research Center, Denver; Loss of tumor suppressor gene expression in canine cancer: sporadic or heritable lesions: The observation that melanoma, lymphoma/leukemia, osteosacoma, and hemangiosarcoma are particularly prevalent in selected breeds of dogs suggests that heritable risk factors play a role in the pathogenesis of these tumors. However, these risks remain undefined at the molecular level. Selected tumor suppressor genes are frequent targets of mutation, and loss of function of genes in at least two distinct pathways that control growth and maintain genetic integrity seems to be a requirement of neoplastic transformation. This loss of function can occur by inactivation of both alleles in somatic cells. Alternatively, a similar phenotype may result from inheritance of a mutant (non-functional) allele followed by inactivation of the wild type allele through a loss of heterozygosity. In the latter case, the cancer risk on an individual is substantially increased; tumors tend to appear at the younger age, and may arise on multiple sites. They have sought to identify tumor suppressor genes that might be targets of inactivation in sporadic canine tumors, but that also might be candidates for increased heritable risk in defined breeds or families. They have studied the role of kinase inhibitors (p21 and p16) as keystone regulators of cell cycle progression, as well as PTEN, an inducer of apoptosis (cellular aging resulting in death of the cell) provided the rationale to examine the frequence of inactivation of the corresponding genes in canine tumor. Evaluation of pilot samples from lymphoid, osteogenic, melanocytic and vascular tumors, showed that loss of expression of these genes occurred in approximately 50% of the tumors. This suggests that these genes may be frequent targets for inactivation in canine cancer. There current efforts seek to examine the possibility that loss of expression of these genes in the tumors may be secondary to inheritance of a mutant allele followed by loss of heterozygosity, and to determine if the inheritance pattern is present in additional affected or unaffected family members. Furthermore, they plan to evaluate whether loss of expression (or function) of these genes might predict poor response to conventional therapies and decreased survival times.

Dr. Roy Levine; College of Veterinary Medicine, Cornell University; Tumor suppressor genes in canine osteosarcoma: Osteosarcoma (OS) is an example of a highly metastatic tumor in which the tumor cells' response to environmental signals is disrupted. Tumor suppressor genes are responsible for mediating the actions of external and internal negative regulators by regulating cell cycle progression and inducing apoptosis (cellular aging resulting in death of cells). They studied p53, Rb and PTEN tumor suppressor genes. In an OS cell line lacking p16 and p53 but expressing RB, induction of p16 or p53 results in a reduction of phosphorylated Rb, increased cell size, and growth arrest. No evidence of apoptosis is apparent at 96 hours post-induction. In contrast, the induction of p53 in an OS cell line lacking p53 and Rb, results in apoptotic cell death. To determine whether inactivation of PTEN also plays a role in the parthogenesis of canine OS, they studied its expression in canine OS cell lines and tumors. In conclusion their results indicate that PTEN is mutated and/or down-regulated in a high percentage of canine OS cell lines and tumors and, along with p53 and Rb family genes, likely plays an important role in the pathogenesis of the disease.

Dr. Barbara E. Kitchell; University of Illinois, Urbana, IL; Telomerase and canine cancer: The search for effective anticancer strategies in the 1990's lead to the discovery of a small core of common genetic defects and alterations in normal regulatory pathways that result in malignant transformation of cells. One of these requisite conditions is cellular immortalization, usually due to the upregulation of an enzyme called telomerase. Telomerase is a ribonucleoprotein that normally acts as a reverse transcriptase to extend telomeres during embryonal development, in germ cells, and in select populations of stemcells in the body. Eukaryotic cells must maintain telomeres, the non-coding hexanucleotide repeats at chromosome ends, in order to retain chromosomal integrity. Virtually all cancers express the telomerase enzyme as a primary defense against the progressive degradation of the chromosome ends that occurs during every round of cell replication. Thus, inhibition of telomerase presents an attractive anticancer strategy. Traditional preclinical murine models of cancer treatment strategies may prove inadequate or inappropriate to evaluate the positive and negative impacts of telomerase inhibition on patient health. For this reason, we have pursued the development of a large, outbred animal cancer model, the tumor-bearing dog, for evaluation of the concept of telomerase inhibition. We have characterized the presence of telomerase activity in spontaneous canine tumors and normal tissues, and have determined that virtually the same tumor types express telomerase with the same relative frequency in both canine and human cancer patients. Overall, in canine cells, the telomerase assay in our laboratory has 86% sensitivity and 95% specificity for malignancy. They hope to characterize the impact of telomerase inhibitors in tumor bearing dogs to better define the positive and negative impacts of such

therapy in advance of human testing, as well as to provide a novel therapeutic avenue for companion dogs with cancer.

Dr. Stuart C. Helfand; University of Wisconsin, Madison; Targeting the tumor microenvironment with Interleukin-12: Directing cancer treatment towards therapeutic molecular targets is a strategy that offers a number of potential advantages,
including increased specificity, increased efficacy, and decreased toxicity. They have developed an approach to deliver interleukin-12 (IL-12) to the tumor microenvironment by targeting an adhesion molecule, avb3 integrin, that is preferentially expressed by endothelial cells comprising newly formed tumor vasculature. IL-12 is one of the most potent immunostimulatory cytokines, having prominent effects in the generation of cell-mediated immune responses important in cancer immunotherapy. It also induces a number of chemokines that mediate potent antiangiogenic effects. The dual actions of IL-12 make it a cytokine of interest for use in the cancer clinic, although induction of high levels of interferon–alpha following its systemic administration has caused toxicity in human patients. To avoid this problem, they engineered a fusion protein (mouse recombinant IL-12 vascular homing peptide, mrIL-12vp) consisting of the peptide ligand for avb3 integrin, coupled to mouse recombinant IL-12. Because fusion protein homes to avb3 expressed by dividing endothelial cells in the tumor microenvironment, IL-12 is available to activate immune cells within the tumor and to trigger antiangiogenic responses concurrently. Using a murine assay to assess *in vivo* angiogenesis, mrIL-12vp mediated potent inhibitory effects on angiogenesis at non-toxic concentrations that were well below those required to show antiangiogenic activity of mrIL-12 alone. Targeting IL-12 with the fusion protein appears to be a means to capitalize on several activities of IL-12 that are important for cancer therapy while reducing toxicity associated with this cytokine. Development of this fusion protein offers new opportunities to exploit immune approaches for inhibiting angiogenesis in cancer.

Dr. Mark W. Dewhirst; Duke University Medical Center, Durham, North Carolina; IL-12-mediated thermoimmunogene therapy in combination with fractionated radiotherapy: Interleukin 12 (IL-12) has shown strong antitumoral effects in numerous pre-clinical studies and appears to act synergistically with radiation in murine tumors. The major impediment to its clinical use has been its systemic toxicity. While using

intratumorally injected viral gene therapy vectors encoding IL-12 reduces systemic side effects substantially, elevated systemic transgene levels are still observed because adenovirus can reach the circulation and infect organs for which these viruses have high tropism (e.g. liver). Further restricting IL-12 expression in the tumor is therefore desirable in a combined radiation and adenovirus mediated cancer gene therapy regimen.

Hyperthermia-regulated gene therapy was tested in a melanoma cell line. For hyperthermic gene therapy, an adenoviral vector coding for IL-12 under the control of the promoter of a human heat shock protein was used. The combination of IL-12 gene therapy with hyperthermia yielded significant antitumor effects, as did radiotherapy alone. However, the best response occurred when radiation was added to the regimen. The improved effect was achieved without apparent systemic toxicity. When used as a single dose, applying IL-12 gene therapy after completion of radiotherapy appears to be beneficial.

Dr. Steven Dow; National Jewish Medical and Research Center, Veterinary Cancer Specialists, Denver, Colorado; Gene therapy for cancer in dogs: Dr. Dow and associates have studied both local and systemic cancer gene therapy approaches to treatment of cancer in dogs. To trigger intense local T cell immune responses, bacterial superantigen genes and cytokine genes were injected intratumorally, using lipid-DNA complexes. They found that local tumor gene therapy with the 2 genes could trigger tumor regression and systemic antitumor activity in dogs with stage III melanoma. More recently, systemic (intravenous) gene delivery has been investigated in dogs with cancer, using intravenous infusions of lipid-DNA complexes. The objective of this approach has been to target gene delivery and expression to the vasculature of the lungs or tumors themselves in order to induce antitumor immunity or inhibit tumor angiogenesis. Intravenous IL-2 gene delivery was evaluated in dogs with stage IV osteosarcoma lung metastases. They found that systemic cytokine gene delivery elicited marked non- specific immune activation and antitumor activity in animals with osteosarcoma and resulted in a significant increase in overall survival time compared to historical control animals. In an ongoing study, significant inhibition of tumor angiogenesis in dogs with soft tissue sarcoma has been observed following intravenous endostatin gene delivery. From these studies, they conclude that local tumor gene therapy may be effective for treatment of some primary tumors in dogs. In addition, preliminary data also indicated that genes can be delivered safely intravenously in tumor-bearing dogs and that systemic

non-viral gene therapy may be an effective new approach to the management of tumor metastases.

Dr. Angela E. Frimberger; Roger Williams Hospital, Providence, Rhode Island; Bone marrow transplantation-based experimental therapies for canine lymphoma patients: Although standard-dose combination chemotherapy for canine lymphoma provides a high rate of clinical remission, relapse after a median 10 to 12 months is the rule and eventually lymphoma is almost universally fatal to affected dogs. High dose chemotherapy with hematopoietic stem cell (HSC) support, or bone marrow transplantation (BMT), has become increasingly important in the treatment of a variety of cancers in people. Because chemotherapeutic drugs exhibit a dose-response relationship, increased dose intensity should result in increased antitumor effect, and strong clinical evidence supports this. However, the clinical utility of dose intensification is limited by the toxicity of the drug regimen. Although under certain circumstances BMT may offer a hope for cure for both humans and dogs that could not be achieved using standard chemotherapy, the toxicity and risk associated with such aggressive treatment are high. In recent years investigators have focused on submyeloablative BMT. The rationale for this approach is to increase the dose intensity above standard, thereby improving antitumor efficacy, without incurring the level of toxicity associated with myeloablative regimens. It has recently been established that myeloablation is not needed for allogeneic engraftment to occur. Thus, submyeloablative allogeneic transplantation specifically for the purpose of triggering a graft versus tumor (GVT) effect has become the subject of intense study in human oncology, and early clinical results are encouraging in terms of both tolerability and efficacy.

The main toxicity of these regimens, as expected, is graft versus host disease. A minimally toxic, nonmyeloablative protocol for creation of allochimerism in normal dogs has been determined and published. They have adapted this protocol for use in dogs with any refractory malignancy and are currently recruiting candidates. They expect to find that, as in humans with lymphoma, nonmyeloablative allogeneic marrow transplant can produce chimerism with minimal toxicity. Further, we hope to show that allochimerism will result in significant GVT, resulting in overall improvement in therapeutic outcome for canine lymphoma.

Dr. George Brewer; University of Michigan, Ann Arbor, Michigan; Antiangiogenic cancer therapy in mice, humans and dogs with anticopper drug tetrathiomolybdate: Over the last couple of decades, they have developed two new anticopper drugs for therapy of Wilson’s disease, an inherited disorder of copper accumulation and copper

toxicity. They have utilized zinc, which induces intestinal cell metallothionein, blocking copper absorption. Zinc is excellent for life-long maintenance therapy in Wilson's disease, with very little toxicity. They have also developed tetrathiomolybdate (TM), which is extremely fast acting, for initial therapy of Wilson's disease patients. TM works by forming a stable, copper-TM-protein complex, rendering copper non-toxic and unavailable for cellular uptake.

Also during the last couple of decades, an important role for copper in angiogenesis has been established. Copper stimulates angiogenesis in the rabbit cornea model, and copper deficient rabbits have reduced angiogenesis. Angiogenesis is required for tumor growth, including growth of metastases. Although copper is an essential element, they hypothesize that its growth and angiogenic promoting effects occur primarily at levels higher than those required for basic cellular functions. That is, they postulate that there is a "window" of body copper status to which copper levels can be dropped, inhibiting angiogenesis, without interfering with critical requirements for copper. Because TM is a remarkably potent, fast-acting, non-toxic, anticopper agent, they elected to try it as an antiangiogenic therapy for cancer. They initiated a phase I/II human clinical trial of TM therapy of a variety of advanced and metastatic cancers, and have achieved encouraging results of efficacy and low toxicity. A total of 40 human patients have been entered, and 18 of these are evaluable by our criteria, which requires that the patient be in the "window" of copper depletion for at least 2 months. All 18 have achieved disease stabilization for longer than two months, with the average being 9.5 months, two (metastatic breast and metastatic chondrosarcoma) for about three years, with six patients still on study.

Most recently, they have initiated a trial of TM in advanced canine cancers, in collaboration with colleagues at the University of California-Davis. After early work to establish dose, they now have two dogs with encouraging early results. They have had disease stability for about two months in a metastatic mammary cancer case, and have overall stability for about three months and disease regression of lung metastases in another dog with metastatic osteosarcoma. In summary, they believe copper levels are an important regulator of angiogenesis, and lowering copper levels offers a new approach to cancer therapy.

Dr. Rachael R. Thomas; Animal Health Trust, Newmarket, Suffolk, England; A survey of recurrent chromosome aberrations in canine lymphoma: Lymphoma (lymphosarcoma) represents the third most common canine neoplasm, and is generally regarded as closely related to forms of human non-Hodgkin lymphoma (NHL). Human NHL demonstrates a range of

both generalized and subtype-specific, non-random chromosome abnormalities. A number of these aberrations have been correlated with disease progression and response to therapy, aiding the accurate diagnosis, prognosis and appropriate therapy selection for each subtype of human NHL.

At present, the prognosis for canine lymphoma is generally guarded, although a proportion of cases is highly responsive to appropriate chemotherapy. A greater understanding of the underlying mechanisms of dog lymphoma, particularly subtype-specific characteristics, is therefore required. As with human NHL, there is evidence to suggest that dog lymphomas of B-cell origin display fewer chromosome aberrations, and are more responsive to chemotherapy, than those of T-cell origin. Previous cytogenetic studies of dog lymphoma were severely limited by difficulties in accurate chromosome identification and characterization of aberrant karyotypes; however, we have now made major advances in the development of molecular cytogenetic techniques and resources for the dog that allow these limitations to be largely overcome.

Their group is performing an ongoing cytogenetic analysis of dog lymphomas in order to identify consistent chromosome aberrations, to correlate these with the clinical course of the disease, and in turn to enable differentiation of specific lymphoma subtypes. Through our dog-human comparative genomics studies, we aim to determine whether there is an evolutionary link between the genetic basis of lymphoma in these two species, as well as generating additional prognostic indicators that may influence the decision to treat the animal, and the form that treatment takes.

The approaches used in their ongoing study was demonstrated by one particularly unusual lymphoma case. A nine year old entire male collie x retriever-cross presented with an enlarged prescapular lymph node and a mass under the left mandibular region. A diagnosis of lymphoma was confirmed by histological evaluation of fixed tissue sections, and immunophenotyping demonstrated that 95% of tumor cells expressed both CD3 and CD79a cell markers, indicating B- and T-cell co-expression. Co-expression is extremely rare in human lymphoma, and to our knowledge has not been described in the dog. Comparative genome hybridization analysis detected loss of dog chromosomes 11, 30 and 38, and gain of chromosome 36, which was supported by direct analysis of tumor metaphases using chromosome specific probes representing each of these four chromosomes. No recurrent chromosome translocations were detected.

Dr. Elizabeth A. McNeil; Colorado State University, Fort Collins, Colorado; Increase prevalence of gastric cancer in Chow Chows: Gastric cancer is an infrequent diagnosis in dogs, comprising less than 1% of canine malignancies according to the literature. A breed predisposition in Belgian shepherds has previously been hypothesized, but has not been investigated epidemiologically. In recent years, they noticed an apparent over-representation of Chow Chows with a diagnosis of gastric adenocarcinoma. To determine the relative frequency of gastric adenocarcinoma in Chows compared to dogs of other breeds and to describe the clinical and histologic features of gastric cancer in Chows, they conducted searches of the databases of the Colorado State University Veterinary Teaching Hospital (CSU-VTH) and the Veterinary Medicine Database at Purdue University (VMDB). From each source, they determined the proportion of dogs in general, and Chows specifically, diagnosed with gastric carcinoma. At the Colorado State University Veterinary Teaching Hospital (CSU-VTH) 12% of gastric carcinomas were diagnosed in Chows. The diagnosis of gastric carcinoma was made in 1.3% of Chows presenting to the CSU-VTH, 10 times the frequency determined for the entire canine population. For the VMDB records approximately 16 percent (32 cases) of the gastric carcinomas occurred in Chows. The prevalence odds ratio for gastric carcinoma in chows in this population was 20.2 (95% confidence interval = 13.6 - 30). These results strongly suggest a breed predisposition to gastric cancer.

The etiology of gastric cancer in dogs is not yet understood. In humans, both environmental (consumption of nitrates and salted meat, deficiency of micronutrients and antioxidants, *Helicobacter pylori* infection) and genetic (racial and ethnic differences, familial occurrences) factors are thought to contribute to gastric carcinogenesis. Their results suggest that genetic influences may also dictate gastric cancer susceptibility in dogs.

Ms. Rhonda Hovan; Bath, Ohio; Canine cancer, a breeder's perspective: The goal of this presentation was to present some ideas of methods to bridge the gap between researchers and breeders – to the benefit of both, and ultimately, to the benefit of the dogs. The basis reference to breeder in this presentation was to someone who has a long term, committed, sincere interest in the welfare of their breed. Most of the activities of the dog breeder arise from one premise: breeders are also pet owners. Therefore, most breeders consider their dogs as part of their extended family, and they want them to have a good life. But despite everything that they do for their dogs over many years, many of them still die from major diseases affecting their breed, including cancer. Researchers believe that something can be done to improve cancer statistics, and most researchers are already helping to make that happen. Yet the dog breeders and fanciers who could and should be the researcher's natural allies are still sitting on the sidelines. The presenter feels that many are not supporting nor participating in research projects because, for the

most part, they do not know researchers, what researchers are doing, how to find them, what breeders need from them, or how researchers can help.

Connecting with Breeders and Fanciers: Typically, news that a dog has cancer is delivered to the owner by the local general veterinarian. At this point, some owners investigate no further. They and their veterinarian elect to simply keep the case in house, and these dogs will not be available to research. But there are other owners who react to the news by seeking information and connections to help their dog. This breeder group is reachable by researchers needing to recruit affected dogs into their study.

Most serious breeders and exhibitors are members of their national Parent Breed Club, and this should be a productive source of contacts. Many researchers already have a relationship with some Parent Clubs through the Canine Health Foundation. Perhaps some Breed Clubs are even contributing funding toward research. Frequently, however, these relationships are not well developed, and much potential support remains untapped.

Every Parent Club has some sort of Health Liaison of Health & Genetics Committee, and the Canine Health Foundation can provide the contact information. Most Breed Club's publish magazines, and editors are generally willing and eager to include articles about health issues concerning their breed. It is usually time well invested fro researchers to prepare an article, directed at breeders and fanciers, suitable for these publications.

In addition, most Parent Clubs maintain web sites. These can be valuable sources of outreach in two ways that Club magazines are not: first, they are much more immediate and timely ways to publicize new study information; and second, web sites are accessible to pet owners who are not competition oriented, and who are not members of the Club. The Club Health Liaison person may also be of assistance in another way. A Club Liaison person functioning as a contact may be a time saver by insulating the researcher in at least this first step, from direct contact with owners.

Depending upon the nature of the research and the climate within the Breed Club, breeders are sometimes reluctant to acknowledge health difficulties in their line. Even statements of confidentiality don't seem to offer enough assurances for some to be moved to participate. Other times, simple apathy or lack of awareness, can be equally as difficult to overcome. In nearly every breed, there are certain persons with the influence to help motivate their peers to rally to a cause. These are usually long-time, respected breeders, who are willing to take a public stand. Not surprisingly, breeders whose dogs have been affected by the cancer in question are often the most effective spokesperson.

Causes, Cures, and Prevention: Some dog breeders consider health issues as being primarily within the control of breeding decision. Other breeders and concerned about protecting their investment in their own personal breeding stock. The first type of breeder tries to identify a certain dog or line that is responsible and the second tends to blame environmental chemicals or food contaminating chemicals as the problem.

Concerning cancer and other canine diseases, researchers should state very clearly and repetitively, whether they are referring to inherited genetic mutations, versus sporadic genetic mutation, or when that status is unknown. Breeders tend to hear only the work "genetic," and their assumptions take off from there. To most, "genetic" means inherited. And when the ensuing finger pointing, defensiveness, and suspicions within the breeding community are not helpful to anyone, and may even cause some breeders to be reluctant to participate in research projects.

For those persons whose dogs currently have cancer, the help they are most interested in is a cure. Second to that are good treatment options, defined as being affordable, maintaining quality of life, and offering significant extension of life. But treatment is not the long term solution that breeders seek. Treatment would not help the breeder protect the next generation of dogs. Breeders need to know as much as possible about how to identify genes that put their dogs at higher risk. Equally as important, breeders need to know, if possible, when a disease is not due to heritable factors.

Research Web Sites: Many dog breeders do not find out about research studies at the time their dogs was eligible. It is currently difficult to find on the Internet active cancer studies that are recruiting cases. It would be most helpful if the canine cancer research community would organize, produce, and publicize a comprehensive canine cancer research Internet site.

Dr. Todd L. Towell; University of Wisconsin, Madison, Wisconsin; Efficacy and safety of a novel canine intratumor immunotherapy product: The safety and efficacy of a liposome formulated plasmid DNA encoding *Staphylococcus aureus* enterotoxin A (SEA) superantigen and canine interleukin-2 (IL-2) (L-SEA/cIL-2) were evaluated in two studies supported by Heska Coporation. In the first study, the acute safety of a single injection of L-SEA/cIL2 was assessed in 16 dogs with a variety of spontaneous tumors. Dogs were observed immediately post injection and every 6 hours for 36 hours. No adverse reactions were noted in any dog. Rectal body temperatures and heart rates were variable and no clear trends emerged. No clinically significant abnormalities were noted on hematology or serum chemistries. Demonstration of gene expression was hampered by tissue handling techniques but the results suggest the plasmid DNA was functional and that gene specific mRNA can be transcribed. In the second study, 16 dogs with spontaneous soft tissue sarcomas (STS) were treated with weekly injections of L-SEA/cIL- 2. Efficacy was evaluated by serial tumor measurements following ~12 weekly injections. Complete responses (CR) were seen in 3 dogs, and a partial response (PR) in one dog. Stable disease (SD) was reported in one dog. Progressive disease (PD) occurred in 11 dogs. After

approximately 1 year of follow up, two of three CR, the PR and the SD are disease free. The remaining CR is disease free at 6 months. In conclusion, intratumor administration of SEA/IL2 immunotherapy appears well tolerated and demonstrates anti-tumor activity in dogs with spontaneous STS. The study is now being expanded to more dogs.

Dr. Chand Khanna, National Cancer Institute, National Institutes of Health, Bethesda, Maryland; Identification of genetic determinants of metastasis in osteosarcoma; A comparative approach: Despite advances in the management of osteosarcoma, and other solid tumors, the development of metastasis continues to be
the most significant problem and cause of death for cancer patients. To define genetic determinants of pulmonary metastasis in osteosarcoma they have utilized a cross-species comparative approach (murine, canine, and human) for gene identification and evaluation. This approach allows tissues from pet dogs with naturally occurring osteosarcoma to add value and relevance to the genomics data generated from their in vitro and in vivo metastasis models. Within this approach, they have applied cDNA microarrays to a murine model of osteosarcoma, characterized by orthotopic tumor growth, a period of minimal residual disease, spontaneous pulmonary metastasis, and cell line variants that differ in metastatic potential. Microarray analysis identified 59 genes (out of 3899 printed cDNA probes), that were differentially expressed in replicate experiments between the primary tumors of the more aggressive (K7M2) gene and less aggressive (K12) osteosarcoma models. Ezrin, a gene taken from this narrowed gene list and not previously described in osteosarcoma, was 3 fold over-expressed in the more aggressive and less aggressive osteosarcoma mouse models. Ezrin is associated with linking the cell membrane to the actin cytoskeleton with described functions in motility, invasion, and adherence. The potential relevance of ezrin in osteosarcoma and metastasis was examined in dogs with naturally occurring osteosarcoma. Ezrin mRNA expression in canine osteosarcoma primary tumors and pulmonary metastases was demonstrated. The intensity of ezrin staining in pulmonary metastases samples was stronger than primary tumors. Outcome data from this canine osteosarcoma tissue array suggested that dogs without ezrin staining in the primary tumor trended towards improved median disease free interval than dogs with ezrin staining.

Dr. Peter Vajdovich; Szent István University, Budapest, Hungary; Free radical and antioxidant properties of blood and lymph nodes of healthy dogs and dogs with lymphoma: Recent studies report that oxidative stress caused by oxygen free radicals can inhibit the ability of some chemotherapy drugs to induce apoptosis (cellular aging leading to cell

death). The hypothesis for this study was that decreased sensitivity to free radical damage plays a role in maintaining cell proliferation of tumor cells, while they have a decreased antioxidant defense. They examined the difference between antioxidant parameters in blood and lymph nodes of healthy dogs and dogs with lymphoma. The total free radical concentration, measured by spin-resonance-spectoscopy, was increased by 56%, and the phorbol myristate acetate (PMA)-induced free radical production was reduced by 97% in the lymph node homogenates from affected dogs as compared to that of healthy dogs. These results suggest that protection against free radicals by antioxidant defense mechanism is weaker in dogs with lymphoma than in healthy dogs. The observation that there are greater levels of steady-state free radicals in tumor cells may be due to an increased metabolic rate, while PMA could not induce free radical formation in the cells from malignant lymph nodes may be due to a distinct mechanism.