WHAT’S NEW IN CANCER DIAGNOSIS AND THERAPY
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The Animal Cancer Care and Research Program of the University of Minnesota

The Animal Cancer Care and Research Program (ACCR) was established at the University of Minnesota College of Veterinary Medicine (CVM) in 2007. This program is built as a partnership between the CVM and the Masonic Cancer Center, University of Minnesota to devote the necessary resources to reduce the burden caused by cancer in companion animals and humans alike. The ACCR includes more than 50 faculty members from the CVM and other units of the University of Minnesota Academic Health Center whose shared vision is to create a world where we no longer fear cancer. We believe we will achieve this through rigorous and creative research, training, and translation of discovery from the laboratory to the clinic.

Overview

Diligent clinical research over the past 30 years has allowed veterinarians to adapt or develop protocols to treat companion animals with cancer, providing pet owners reasonable options ranging from palliative care to therapies with curative intent. In addition to adapting protocols using conventional modalities (surgery, radiation, and cytotoxic chemotherapy), we have seen targeted drugs and immunotherapy approaches developed specifically for the veterinary market\(^1\)\(^2\). However, we could argue that basic research has lagged behind these clinical advances. In particular, we have failed to address issues such as tumor heterogeneity in some of the most common cancers we diagnose, and advances to treat highly aggressive tumors such as hemangiosarcoma have been modest. Our laboratory has emphasized these areas, focusing on improved understanding of cancer pathogenesis, development stratification schemes that can improve prognosis and prediction, and applying these to advance innovations in therapy. Here, we will review recent data focusing on canine lymphoma, canine osteosarcoma, and canine hemangiosarcoma, emphasizing practical applications that may be useful to practitioners in both general and specialty practice.

Objectives

1. Recognize that tumors are heterogeneous and that each “named disease” is in fact a collection of many diseases
2. Highlight the importance of accurate diagnostic classification to provide the best treatment for cancer
3. Establish the highest standards for diagnostic testing that will improve relations between the veterinary care team and between the attending veterinarian and the owner
4. Describe how appropriate application of diagnostic tests will help improve patient care and lead to more satisfactory patient outcomes
5. Highlight recent advances in therapeutic development for canine lymphoma, osteosarcoma, and hemangiosarcoma.
**Canine lymphoma – one disease or many?**

Canine lymphoma is a heterogeneous group of diseases that share malignant transformation of lymphocytes as a common property. Several systems have been proposed to classify lymphoma over the past four decades {see 3 for review}, including the modified WHO classification system, which was initially proposed in 2002, but has not yet been universally adopted. The most contemporary WHO classification system for B- and T-cell lymphomas includes approximately 30 subtypes 4. The system combines morphology, topography, immunophenotype, and clinical progression to define these disease entities. In our experience, six subtypes are commonly observed, including diffuse large B-cell lymphoma (DLBCL), marginal-zone lymphoma (MZL), Burkitt and Burkitt-like lymphoma (BL), lymphoblastic T-cell lymphoma (LBT), T-zone lymphoma (TZL) and peripheral T-cell lymphoma - not otherwise specified (PTCL) 3,5. Other studies support the frequency with which these tumor types are observed 4.

Some commonly held myths about lymphoma are especially unnerving. For example, the concept that B-cell tumors are “bad” and T-cell tumors are “terrible” means that veterinarians may be reluctant to treat any dog diagnosed with T-cell lymphoma. The lack of documented prognostic significance has raised doubts about cost:benefit and risk:benefit ratios of the diagnostic procedures needed to assign any sample to its category in the WHO classification. Further, current therapeutic regimens are not tailored for lymphoma subtypes, highlighting both opportunities for improvement and additional reasons for the observed resistance to the expense and effort of classification. We have now shown conclusively that not all T-cell lymphomas carry a poor prognosis 5,6. Indeed, dogs with indolent T-cell lymphomas, such as T-zone lymphomas, have a favorable prognosis and may not require aggressive therapy 7. What is more, aggressive therapy may be contraindicated for these cases, potentially shortening remission times and survival. Our institution is one of several that has instituted recommendations to classify every case of lymphoma as part of our routine practice to provide the best possible information to owners and the best therapeutic options for their pets. To improve access to these services, we used molecular data to develop new tests that can be used to distinguish clinically relevant subtypes in single cell suspensions that can be obtained using fine needle aspiration. This test has been licensed for development and implementation in the diagnostic laboratory setting, and should soon help owners make decisions and improve care and quality of life for dogs with indolent tumors.

However, access to effective therapies is needed for dogs with aggressive subtypes of lymphoma 3. Immunotherapy for lymphoma has revolutionized treatment and outcomes for human patients, and new reagents are becoming available to apply similar strategies for dogs. Indiscriminate use of reagents without scientific validation can bring harm to patients. Thus, we have worked deliberately to ensure that targets for immunotherapy such as the CD20 antigen are expressed by canine lymphoma cells 8, and we and others have developed novel approaches and nurtured partnerships to develop canine-specific reagents with potential for immunotherapeutic applications in the veterinary space 9,15. Our results indicate that the rational and careful application of canine-specific immunotherapy has great potential to improve outcomes for canine lymphoma patients in the near future.
Canine osteosarcoma – one is not as bad as the other?

Osteosarcoma is the most common tumor of bones in dogs; it represents 3-6% of all canine tumors with at least 8,000, and possibly more cases diagnosed each year in the USA. These tumors are highly aggressive, characterized by local invasion and destruction, and by distant metastasis. In general, dogs with appendicular osteosarcoma present at stage 2b (tumor with high-grade histologic appearance outside the periosteum and no detectable metastases). Tumors spread preferentially to lungs more commonly than to bones or other organs. Predictive factors for survival, which are similar for dogs and people, include age at diagnosis; anatomic location and size of the tumor; histologic grade; serum alkaline phosphatase concentrations; and initial response to therapy.

Without treatment, affected dogs usually live <10 weeks. Palliative treatment controls the pain associated with the disease, but does not extend survival. Amputation increases survival to ~5-7 months. The standard of care includes amputation or limb-sparing surgery followed by adjuvant chemotherapy to delay growth of micro-metastases that are invariably present by the time the disease is diagnosed. Gross metastases are seen within 6-10 months in more than 50% of animals that receive standard of care and within three years in more than 90%. The reported range for median overall survival with standard of care is ~6 to ~11 months, with less than 30% of dogs surviving two years and less than 10% of dogs surviving three years.

There is no difference in survival among the three most common histologic tumor subtypes (osteoblastic, chondroblastic, fibroblastic osteosarcoma). While the survival statistics are discouraging, it is useful to compare these data with the results of children with bone cancer (the highest risk group in people). In children, standard of care produces an overall survival rate of ~80%, but event-free survival is lower (~60% at five years) and only ~50% will survive 10 years. This means 20% of children diagnosed with osteosarcoma will not survive five years, as many as 50% may not see the tenth anniversary of their diagnosis, and most have significant morbidity associated with the disease. The timeframe bracketed by these hallmarks in children represents ~10% of an average adult lifetime, which provides a reasonable basis on which to compare the best clinical outcome with dogs that have bone tumors, indicating that, at least at present, the outcomes for osteosarcoma patients, whether human or canine, are rather dismal. Since the disease ultimately leads to death of most patients, affected dogs and dogs at risk (as well as affected children and children at risk) would clearly benefit, respectively, from improved options for treatment and prevention.

We surmised that we could probe the molecular features of this disease to reduce heterogeneity and improve prediction. Considering the similar clinical presentation, we postulated evolutionarily conserved molecular traits for this disease would be present in dogs and humans. Thus, the narrower genetic diversity of dogs would enhance our ability to define biologically and clinically significant traits. Defining genome-wide gene expression profiles allowed us to define two distinct molecular subgroups of osteosarcoma. The clustering defined by this signature was seen repeatedly in three and five unrelated data sets from dogs and humans, respectively, although bone tumors may have more complex behavior in humans than in dogs. Nonetheless, when we consider known differences between canine and human osteosarcoma, such as the age
of disease onset and the palliative vs. curative treatment applied to these species, respectively, the similarities observed in their molecular signatures and associated biological behaviors are remarkable. Despite repeated attempts and the application of numerous algorithms, previous unsupervised analyses failed to segregate samples from intact tumor tissues (i.e., including tumor cells and stroma) from dogs or humans into meaningful groups. Thus, even though the gene expression signature was present in these intact tumor samples from both species, it was masked by stromal signatures can modulate the balance of expression for some of the genes. The significance of this restricted gene list is further underscored by its capacity to segregate independent cohorts into distinct branches, where each branch likely represents a molecular subtype with unique and potentially predictable biological behavior.

Now that we can define a group of dogs with more indolent (or less aggressive) osteosarcomas that are likely to have more favorable response to therapy, the next step is to develop tests that can be used by practitioners in general and specialty practice. Genome-wide gene expression profiling is not practical for clinical cases. It is expensive, labor intensive, and turnaround is not consistent with therapeutic needs. We thus sought to develop additional tests that would be translatable to the needs of commercial diagnostic laboratories and the clinicians who comprise their principal clientele. We have developed two molecular approaches that can be used in productive fine needle aspirate samples, and which can robustly predict biological behavior. As was true for lymphoma, this will eventually help dog owners make decisions and improve care and quality of life for patients with less aggressive tumors.

Yet, access to more effective therapies is needed for dogs with aggressive forms of osteosarcoma. We are addressing this by exploring novel treatments that specifically target cells with aggressive phenotypes. We have recently completed two clinical trials evaluating immunotherapy platforms that were safe and had robust efficacy signals. We also have identified a central pathway that appears to regulate the biological behavior of osteosarcoma. Our ongoing work is testing the hypothesis that deregulation of this pathway can be reversed, at least in part, using combinatorial therapy with FDA-approved drugs that are being used in human clinical trials.

**Hemangiosarcoma – who art thou?**

Hemangiosarcoma is perhaps the most aggressive vascular tumor diagnosed in dogs. Although systematic surveys are not available to obtain accurate estimates of risk and incidence in companion animals, hemangiosarcoma appears to be relatively common in dogs, unlike its counterpart in humans, which is called angiosarcoma and is vanishingly rare (<0.01% of human tumors). Reports of canine hemangiosarcoma are traceable to the early 1960’s in the current medical literature. By the mid 1970’s, Priester had recognized that hemangiosarcoma of the liver occurred 25 to 100 times more frequently in dogs than in humans, and by the late 1970’s, various investigators noted a breed predilection in German Shepherd Dogs in Europe. The breed predilection for hemangiosarcoma was confirmed and extended through various retrospective studies in the US in the 1980’s and 1990’s. Seminal work from Priester and McKay published as an NCI monograph in 1980 provided the first reasonable estimate for the occurrence of hemangiosarcoma in domestic dogs in North America. Splenic hemangiosarcomas accounted for ~0.3% of all tumors. Several of the common breeds we now
associate with this disease, including Boxers and Golden Retrievers, were already overrepresented in the affected population of this systematic survey. Notably, Priester and McKay reported no significant differences in risk between male, female, or neutered animals. Arguably, the larger prevalence of sexually intact animals in that era might provide more accurate numbers to assess the potential effect of neutering on a dog’s risk to develop this disease.

The common primary sites for hemangiosarcoma are the spleen, the right atrium of the heart, the liver, and the subcutis. The pattern of growth involves infiltration into normal tissues surrounding the tumor as well as distant metastasis. Yet, the disease is insidious; that is, it does not cause pain and the rate of growth in the early stages is relatively slow. Dogs harboring even large hemangiosarcomas may show no clinical signs or evidence that they have a life threatening disease. Generally, tumor-associated blood vessels are tortuous and malformed, and blood cells tend to pool in them and clot. The clots prevent blood and nutrients from reaching tumor cells, in turn causing these cells to die. This creates small ruptures in the tumor through which blood may escape into the abdomen, heart sac, chest, or subcutaneous space. Depending on the amount of blood lost, affected dogs may show constitutional signs, including lethargy and weakness, but these are transient and resolve as dogs reabsorb the blood components and make new blood cells. The clinical signs are recurrent, but they also are subtle enough to go unnoticed for some time. Microscopic, if not macroscopic metastasis almost certainly is present in most dogs at the time of diagnosis, and the eventual outcome for dogs with this disease often follows the rupture of a large or rapidly growing tumor, which results in acute, severe hemorrhage, collapse, shock, and death.

The response to treatment is unrewarding. Commonly cited median survival for dogs with splenic hemangiosarcoma (the most common form) treated with the standard of care ranges from 3 to 5 months \(^{26}\), with slightly better survival in dogs that had no gross metastasis at diagnosis and up to ~15-20% surviving >12 months. This is consistent with data from 112 cases seen at our hospital in a ~30-month period between 2009 and 2011. Few sarcomas show the metastatic potential seen in hemangiosarcoma. It also is not rare for sarcomas to be chemoresistant \(^{27-29}\). Combined, the high rate of metastasis and chemoresistance places hemangiosarcoma alongside osteosarcoma as two prototypical canine sarcomas that are essentially incurable using conventional therapies. This has led to numerous controlled studies and anecdotal instances of experimental therapy for this disease. Most studies using combinations of cytotoxic drugs, metronomic dose schedules, autologous vaccines, and sundry immune-based and antiangiogenic therapies have not shown consistent improvement over the accepted standard of care.

Perhaps the resistance is due to the peculiar origin and the insidious natural history of this tumor. Hemangiosarcoma can occur in virtually any organ, albeit it tends to be associated with organs or regions that have complex vascular networks (spleen, heart, liver, lungs, brain, kidney, muscle, bone marrow, and skin). Visceral tumors show similar biological behavior, while cutaneous tumors are relatively indolent and can be curable. Unfortunately, these clinico-pathological observations cannot answer the questions as to whether the tumors show site or substrate specificity and whether they originate from a single cell type. An apparent breed-specific predilection for different topological forms of the disease also has been noted \(^{30}\), suggesting tumors at different sites could have different etiology. On the other hand, these differences may
be driven by other microenvironmental factors that are independent from the cell of origin. Over the past decade, we have developed a resource of hemangiosarcoma-derived cell lines with representation of anatomical and breed diversity, and we have examined phenotypic and molecular commonalities among these cell lines, defining characteristics of cell surface antigen expression, tumor suppressor gene inactivation, and genome-wide gene expression profiles. Two important aspects arising from these data were the observation that hemangiosarcoma cells in isolation (cell culture) have phenotypic properties suggestive of bone marrow ontogeny and specifically of bone marrow derived (mesenchymal?) stem cells. Alone, these data could not distinguish if the tumors themselves arise from a cell in the bone marrow that migrates to a vascular plexus, or if they originate from a bone fide stem cell. The gene expression profiling data offered some clues, however, as the recurrent signature of these genes was associated with enrichment of angiogenic and pro-inflammatory genes, with no evidence of tissue specificity. More recent data show that hemangiosarcoma cells do not show site specificity for growth, but intriguingly, they respond to microenvironment cues and adopt various functions as part of this response. These functions included not only the potential to form anatomically distinct structures or tumors, but also the potential to direct other cells in their environment to do so. Hemangiosarcoma cells also include subpopulations that retain traits associated with cancer stem cells, including self-renewal, chemoresistance, and increased tumorigenicity in vivo. Each tumor may achieve these properties by independently disabling or enabling molecular networks associated with self-renewal and survival. Much work remains to be completed. It is apparent that some or many properties of hemangiosarcoma are dependent on interactions between tumor cells and their local microenvironment. These interactions probably depend on the microanatomy of the niche and the capability of the cells to alter the niche by recruitment or reprogramming of local stromal cells. Local or systemic microangiopathy may precede the disease, but it also may be a consequence of the vascular disruption it creates. Platelets and platelet derived factors, inflammation, and hypoxia are probably key drivers of the disease. Our ongoing studies include development of innovative “home-grown” treatment modalities, as well as genetic manipulation of tumors to highlight potential interventions that can be used to attack their environmental niche and improve outcomes for dogs with this dreadful disease.

References