Advances in Comparative Bladder Cancer Research: Molecular Targets and Profiling in Dogs and Humans

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Brief Overview of Human and Canine Urinary Bladder Cancer.

Urinary bladder cancer is newly diagnosed in >500,000 people worldwide including >80,000 people in the United States each year. The majority of bladder tumors are urothelial carcinomas (UC), also referred to as transitional cell carcinomas. UCs are low grade superficial tumors in 70-75% of people, and high grade invasive UCs (InvUC), often referred to as "muscle invasive bladder cancer", in 25-30% of patients. InvUC invades into the bladder wall, and metastasize to distant sites and become lethal in ~50% of patients. Most of the >17,000 bladder cancer-related deaths in the United States each year are due to InvUC. Research is clearly needed to improve the outlook for people facing InvUC.

Dogs with naturally-occurring InvUC have become an important animal model for cross-species bladder cancer research. Experimentally-induced rodent bladder tumor models often fail to adequately represent InvUC in humans. Canine and human InvUC, however, are very similar in histopathologic features, gene expression including molecular subtypes discussed more below, clinical presentation, aggressive behavior, frequent distant metastasis to lung, liver, etc., and response to chemotherapy. The finding of shared luminal and basal molecular subtypes in canine and human InvUC is especially important as these subtypes have been associated with cancer behavior, treatment response, and the host immune response to the cancer. In one interspecies difference, activation of the mitogen-activated protein kinase (MAPK) pathway is more common in canine InvUC than in human InvUC. In human InvUC, MAPK activation is typically limited to the more aggressive cancer. Interestingly, the cancer in both species develops into similar molecular subtypes.

Many tumor types have been described in the urinary bladder in dogs including UC, squamous cell carcinoma, adenocarcinoma, rhabdomyosarcoma, leiomyosarcoma, lymphoma, hemangiosarcoma, fibroma, and other mesenchymal tumors. InvUC is the predominant malignancy in the dog representing >90% of tumors in most studies. An estimated 40,000 – 60,000 dogs develop InvUC each year in the United States.

A summary of key facts about canine InvUC include:

- The trigone region of the bladder is the most common site, although InvUC can occur in other parts of the bladder, as well as in the urethra, prostate, vagina, ureters, and renal pelvis. Due to field carcinogenesis, it often arises in more than one location.
- Certain dog breeds have a high inherited risk for InvUC including a 20X increased risk in Scottish Terriers, and a 3-6X increased risk in West Highland White Terriers, Shetland Sheepdogs, and beagles when compared to mixed breed dogs. It is likely that closely related breeds are also at higher risk for the cancer.
- The clinical signs of InvUC mimic those of urinary tract infection and other bladder diseases. A definitive diagnosis is made by tissue biopsy and histopathology. Cystoscopy has emerged as a key method for biopsy. Studies of molecularly-based urine for detection of InvUC have had mixed results, and will be further discussed in the presentation.

- Tumor staging is accomplished by physical exam with digital rectal exam, thoracic radiography, and abdominal ultrasonography or CT. It is important to follow a standardized imaging approach to map the bladder lesions in order to track the changes over time.
- While it is usually not possible to cure InvUC in dogs, the cancer can be controlled with therapy allowing most dogs to live months to a year or more typically with good quality of life.
- InvUC therapy can include surgery, radiation therapy, and/or drugs depending on the extent of the cancer present. Drugs are the mainstay of InvUC therapy in dogs. Therapy can be tailored to meet the needs of each dog, and the capacity and desire of the dog owners to pursue treatment for their dog. A conservative treatment is a single-agent nonsteroidal anti-inflammatory drug (NSAID, i.e. cyclooxygenase or "COX" inhibitor) which is associated with a 20% remission rate, 55% stable disease rate, median survival of ~200 days, and one-year survival rate of ~20%. Treatment with intravenous vinblastine plus a NSAID is associated with a 58% remission rate, especially if this is the first treatment given, and a median survival of ~300 days. When different drug protocols are given sequentially, the median survival time extends beyond a year. The best outcomes have been seen in the unusual instance in which the InvUC is in the bladder apex, and is surgically removed, followed by lifelong NSAID therapy. In a small case series, this approach was associated with a median survival of 750 days, with some dogs having no cancer recurrence.
- Published findings provide a list of steps that can be taken to reduce the risk of bladder cancer especially in dogs in high risk breeds. These include avoiding old generation flea control products such as dips and sprays, avoiding lawn chemicals, maintaining healthy body weight and preventing obesity, and consuming vegetables to supplement the dog food.

<u>Examples of Advances in Comparative Bladder Cancer Research</u>.

Comparative bladder cancer research is proceeding on several fronts. Examples of this will be discussed including: (1) research in screening, early detection, and early intervention, (2) molecular subtyping and the potential to personalize care, (3) new treatment strategies, and (4) evolving programmatic support such as the National Cancer Institute's Integrated Canine Data Commons (ICDC).

Screening, Early Detection, and Early Intervention:

There is considerable interest in learning how to detect cancer and intervene earlier in the cancer development process in dogs and humans. Early cancer is expected to respond better to therapy than more advanced cancer. Earlier-diagnosed cancer is associated with less genetic diversity, fewer active drug resistance pathways, more competent host antitumor immune responses, and adequate tumor blood supply to facilitate drug delivery when compared to more advanced cancer. In addition, patients with a lower cancer burden are generally healthier and better able to handle cancer therapy. In dogs, the very high breed-associated risk for InvUC provides an unmatched opportunity to study early detection and early intervention. PRELIMINARY DATA from an early detection / early intervention study in Scottish Terriers provides evidence for the value of this approach. Briefly, 120 Scottish Terriers > 6 years old were prospectively screened for three years by means of ultrasonography, and urinalysis with urine sediment exam at 6-month intervals. Cystoscopic biopsy was performed in dogs with positive screening tests. To evaluate early intervention, dogs with biopsy-proven cancer were enrolled in a trial of the COX inhibitor, deracoxib (Deramaxx, Elanco, Greenfield, IN). The results were intriguing. Biopsy-confirmed bladder cancer was detected in 27% of the 120 Scotties mostly consisting of InvUC, some starting as dysplasia. These dogs had no clinical signs, i.e. no "symptoms" or outward evidence of any urinary tract problems.

A suspected anatomic variant in the form of a nonprogressive bladder apex nodule was also observed in 6% of the Scotties (Heng et al., Vet Radiol Ultrasound, 2022). The deracoxib remission rate was also very promising in this early cancer setting, with ~40% of the dogs having complete or partial remission of their cancer. This compares favorably to the 17-25% remission rate with single agent NSAIDs in dogs with "symptomatic" cancer in earlier studies. Transcriptomic signatures provided relevance to human InvUC including the expression of druggable targets such as EGFR and the PI3K-AKT-mTOR pathway which are also found in human bladder cancer. Additionally, intriguing differences in gene expression were noted between early and later canine tumors. Further research in this area is clearly justified.

Molecular Subtyping and the Potential to Personalize Care:

A recent discovery in bladder cancer research is tumor RNA expression patterns that segregate InvUC into molecular subtypes, with cancer behavior and treatment responses differing between subtypes. These subtypes were first identified in transcriptomic data from human breast cancer, and more recently have been found in human and canine InvUC. At the highest level, human InvUC is divided into luminal and basal subtypes, with further subgrouping within each of these. There is strong agreement across studies for subtype classifications, although different terminology has been used by different research groups. In canine InvUC, luminal and basal subtypes have been identified using a 60-gene class prediction model, and these subtypes closely align with those in humans. In a study of InvUC from 56 dogs, 29 tumors were luminal, and 27 tumors were basal subtype. Basal tumors were associated with greater immune infiltration, progression signatures in RNA-seq analyses, more advanced clinical stage, and earlier development of distant metastases, similar to that in humans. Luminal tumors were strongly associated with dogs in high-risk breeds for InvUC, and less advanced clinical stage. Work is ongoing to probe these findings to understand the processes involved in the development and progression of bladder cancer, and to study the predictive value for molecular subtype in disease progression and treatment response.

New Treatment Strategies:

New treatment strategies involving drugs are progressing on at least three fronts: (1) making better use of existing drugs, (2) applying new targeted drugs, and (3) developing new immunotherapies.

Examples of making better use of existing drugs are provided by the use of NSAIDs as anticancer agents in dogs with bladder cancer, and the use of vinblastine which was previously administered mostly to dogs with mast cell tumors and lymphoid malignancies. Multiple groups are working on new immunotherapies for dogs, and these should be forthcoming in the not too distant future.

An example of the promise of new targeted drugs comes from a trial of vemurafenib in dogs with InvUC. Vemurafenib blocks $BRAF^{V595E}$ (the dog homologue of human $BRAF^{V600E}$) and stops aberrant MAPK signaling resulting from the mutation. Briefly, a phase I/II clinical trial of vemurafenib was conducted at Purdue University in dogs with biopsy-confirmed InvUC harboring the $BRAF^{V595E}$ mutation. The phase I part of the study defined the maximum tolerated dose (MTD) and adverse event profile. The phase II part of the trial was done to estimate the level of anticancer activity of vemurafenib. In results, the MTD was 37.5 mg/kg BID orally, with anorexia being the most common adverse event. Increase in alanine amino transferase was commonly observed, but there was no evidence of hepatic dysfunction. At the MTD, partial remission occurred in 9 of 24 dogs (38%), with a median progression free interval of 181 days (range 53-608 days). It is important to note that this remission rate occurred in dogs receiving

vemurafenib as a true single agent, i.e. not combined with a NSAID. When chemotherapy agents are given as true single agents to dogs with InvUC, the remission rates are <25%. In analyses of RNA-seq data from cystoscopic biopsies collected before and during therapy, and at relapse, the most consistent changes included significantly enhanced immune signatures, i.e. “immune hot”, during therapy, and an “immune cold” state at relapse. This sets the stage for further cross-species research to improve *BRAF* targeted therapies. Vemurafenib will also be an excellent addition to canine InvUC therapy once it is available in an affordable manner. Other targeted drugs have also been evaluated in dogs with InvUC including palladia and lapatinib.

The National Cancer Institute’s Integrated Canine Data Commons (ICDC):

The ICDC is an exceptional resource for canine genomics research. The ICDC which went public in 2020, is sponsored by the National Cancer Institute with the main goal to collect and share canine cancer data with the community as part of NCI’s Cancer Research Data Commons (CRDC). Briefly, canine study data including genomic sequencing, pathology, clinical features, treatment, case outcomes, and other types of data and images can be deposited in the ICDC. The data can be analyzed in a cross-species manner alongside human cancer data from the CRDC using Cloud Resources. More information is available at: https://caninecommons.cancer.gov/#/ .

Supplementary Reading Material.

Canine Bladder Cancer and Dog-Human Comparison Studies

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