DOI: 10.1111/vco.12784

Veterinary and Comparative Oncology WILEY

ABSTRACTS

SENSITIZATION OF MACROSCOPIC PULMONARY OSTEOSARCOMA METASTASES TO CONVENTIONAL DOXORUBICIN THERAPY WITH SYNCHRONOUS PULSATILE ORAL PAC-1

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Introduction: Progressive pulmonary metastases are expected for dogs diagnosed with osteosarcoma (OS) when localized treatment(s) are effective. Less than 5% of dogs that have measurable pulmonary metastasis will benefit from chemotherapy. Procaspase-3 (PC-3) is overexpressed in a variety of malignancies, including canine OS, and can be targeted with PAC-1 to enhance tumour-killing effects of different chemotherapy drugs. Here, we investigate the combination of PAC-1 and doxorubicin for management of measurable OS pulmonary metastases.
Methods: PC-3 expression is evaluated in canine OS patient samples and OS cell lines by western blot and/or IHC. In vitro combination studies include cytotoxicity assays, apoptosis assessment, PARP cleavage and biomass assessment. Metastatic murine models using luciferase-expressing cell lines are utilized for serial evaluation of single-agent and combination treatment. In an ongoing prospective clinical trial, dogs with radiographic evidence of pulmonary metastases are enrolled to assess the cytoreductive efficacy of PAC-1 and doxorubicin treatment.
Results: PC-3 is expressed by all OS cell lines and patient samples. In vitro experiments and murine models demonstrate favourable trend in combination treatment over single-agent activities. Of the current 12 dogs, response outcomes include 4 SD (>8 weeks), 1 PR and 1 CR resulting in 50% BRR and 16.7% ORR.
Conclusion: OS cells express PC-3 and this can be targeted using PAC-1. In vitro and murine models suggest combination therapy is superior to historical single-agent treatment. The observed ORR and BRR suggests combining PAC-1 and doxorubicin treatment has potential to improve the management of measurable OS pulmonary metastases in dogs.
Funding Information: National Cancer Institute: R01 CA120439-09. PuppyUp Foundation.

INDIVIDUALIZED CHEMOTHERAPY DRUG DOSE ESCALATION IN CANINE LYMPHOMA

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Introduction: We aimed to determine the ability to escalate drug doses in a 15-week CHOP protocol. We hypothesized that at least 50% of dogs would be successfully escalated in at least one drug. Secondary endpoints included objective response rate (ORR), progression-free interval (PFI) and overall survival time (OST).
Methods: 30 dogs with newly diagnosed lymphoma were prospectively treated with a 15-week CHOP protocol. The first cycle was administered at standard doses. Drug doses that did not cause dose-limiting adverse effects (AEs) were increased using a standardized dose escalation protocol. AEs and response to therapy were assessed using VCOG criteria.
Results: Of 30 patients, 23 had the opportunity to dose escalate. Of those, 78% were successfully escalated (18/23). Vincristine was successfully escalated to a minimum of 0.8 mg/m^2 in 11 patients, cyclophosphamide was successfully escalated to a minimum of 300 mg/m2 in 16 patients and doxorubicin was successfully escalated to a minimum of 35 m/m^2 or 1.4 mg/kg in nine patients. Seven patients (23%) were hospitalized at least once. Neutropenia was the most common dose-limiting toxicity for all drugs. 13/30 patients had T cell lymphoma. Nineteen patients achieved a CR, and 11 patients experienced a PR. The median PFI was 171 days (203.5 for B cell, 83 for T cell). The median OST was 254 days (260 for B cell, 222 for T cell).
Conclusion: Drugs in the CHOP protocol can often be escalated safely, with manageable AEs.
Funding Information: This study was funded by the Eldred Foundation.

INVESTIGATING THE DEPENDENCY OF METABOTROPIC GLUTAMATE RECEPTOR 1 SIGNALLING FOR SUSTAINING RAPID PROLIFERATION OF HEMANGIOSARCOMA CELLS

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Introduction: Canine splenic hemangiosarcoma (cHSA) is a highly malignant solid tumour that results in near universal fatality. Historically, cytotoxic therapies have been used to minimize residual disease following surgery and yet survival times in these dogs remain disappointing. An alternative strategy that has received limited attention is the exploration of metabolic vulnerabilities that might exert cytostatic effects; reducing cHSA proliferation and progression. The phenomenon of glutamine addiction has been documented in several human cancers, whereby glutamine is a valuable anaplerotic resource utilized in several metabolic pathways. Tumour dependency on glutamine becomes so great that its removal induces cellular senescence and death. Metabotropic glutamate receptor 1 (mGluR1) is a G-coupled protein receptor overexpressed in several cancer types and participates in an autocrine/paracrine feedback loop that promotes cancer cell proliferation. In this study, we seek to understand the influence of glutamine metabolism and mGluR1 inhibition on canine hemangiosarcoma.

Methods: Six cHSA immortalized cell lines and 36 cHSA tumour and metastatic mass biopsies were assessed for mGluR1 expression. Cell line sensitivity to two mGluR1 small molecule inhibitors was evaluated by cellular proliferation assays.

Results: All cHSA cells and biopsy samples express mGluR1. Proliferation in all treated cell lines is reduced when subjected to one of two mGluR1 inhibitor molecules, with an average IC50 of 62.43 (mu)M and 56.49 (mu)M.

Conclusion: Expression of mGluR1 is detected in cHSA immortalized cell lines and biopsy samples. All tested cHSA cell lines tested experience reduced proliferation when treated with mGluR1 inhibitor molecules.

Funding Information: Funded by VCS Resident Research Grant.

PROSPECTIVE EVALUATION OF THE FAECAL MICROBIOME IN DOGS WITH CANCER RECEIVING DOXORUBICIN CHEMOTHERAPY

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Introduction: Reduced faecal microbial diversity has been associated with cancer development, treatment response and treatment tolerability through its interaction with the gastrointestinal tract and the immune system in humans. Assessing the faecal microbiome may be a useful tool for helping predict treatment outcomes and side effect profiles in dogs with cancer. We aimed to evaluate changes in faecal microbial diversity after a single dose of doxorubicin chemotherapy and correlate these changes with clinical parameters.

Methods: Fifteen dogs with spontaneously occurring cancer were prospectively enrolled, and stool samples were collected on days 0, 4, 7 and 21 after doxorubicin chemotherapy. The faecal microbiome was analysed via 16S rRNA amplicon sequencing as previously described. Treatment-induced differences in alpha and beta diversity were determined through comparison to data from healthy controls ($n = 27$) using a repeated measures ANOVA and PERMANOVA.

Results: Dogs with cancer receiving doxorubicin had decreased faecal microbial diversity when compared with healthy controls at baseline and at all study time points ($p = .0007, .001, .0001, .0006$). Alpha and beta diversity did not significantly change in dogs throughout a cycle of doxorubicin ($p = .194$ and $.371$). Samples pre-treated with antibiotics were significantly less diverse (alpha and beta diversity) than untreated samples ($p = .0504, .006$ respectively). Gastrointestinal signs were not associated with significant changes to the faecal microbiome during treatment.

Conclusion: The faecal microbiome of healthy dogs and dogs with cancer receiving doxorubicin chemotherapy is relatively stable over time, but dogs with cancer and those receiving antibiotics have reduced microbial diversity compared to healthy dogs.

Funding Information: This study was funded by a 2019 VCS Resident Grant.

INTERROGATING THE CLINICAL IMPLICATIONS OF SOMATIC MUTATIONS IN CANINE SPLENIC HEMANGIOSARCOMA

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Introduction: Canine hemangiosarcoma (HSA) is an aggressive cancer of endothelial cells typically associated with short survival times. Understanding the genomic landscape of canine HSA is critical to develop more effective therapeutic strategies. Recent studies indicate that several key mutations such as those involving PIK3CA are found in HSA. The objective of this study was to leverage a highly curated large population of dogs with splenic HSA to determine whether specific somatic mutations are associated with metastasis at presentation and/or overall survival time.

Methods: Splenic HSA tumour samples from 99 dogs were analysed using the next-generation sequencing panel from the FidoCure® Precision Medicine Platform. Patient signalment, presence of metastasis at diagnosis, doxorubicin treatment and overall survival time were evaluated. The incidence of specific mutations and their relationship to outcome were assessed.

Results: Metastasis at diagnosis was present in 29.2% of patients and negatively correlated with median survival time (136 vs. 260 day, $p = .022$). Interestingly, treatment with doxorubicin did not improve outcome in this population. TP53 mutation was common (40%) and was associated with shorter median survival time (147 vs. 311 days, $p = .054$). Mutations were also identified in PIK3CA (15%), NRAS (13%) and PTEN (5%) although no correlation with survival time was noted.

Conclusion: These data suggest that TP53 mutation is associated with a poor prognosis in dogs with splenic HSA and that the use of doxorubicin may not improve outcome. Additional analysis of this patient cohort is ongoing, including the contribution of several other novel somatic mutations and their relationship to disease biology.

EVALUATION OF ACCIDENTAL ANTHRACYCLINE OVERDOSES IN 16 DOGS

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Introduction: Many case reports of chemotherapy overdoses (ODs) exist in both human and veterinary literature, though anthracycline ODs have been uncommonly described.

Methods: This study was performed as a multicentre retrospective analysis. The American College of Veterinary Internal Medicine oncology and internal medicine listservs were solicited for cases in which a chemotherapy OD occurred.

Results: Thirty-one chemotherapy OD submissions were collected. Of these, there were 16 canine anthracycline ODs, including 12 mitoxantrone and 4 doxorubicin. More anthracycline ODs occurred secondary to an error in chemotherapy preparation ($n = 9$) than due to a dose miscalculation ($n = 7$). Most ODs were identified immediately after administration ($n = 11$) and supportive care was initiated. In the remaining patients, supportive care was initiated when clinical signs appeared.

The overall median overdose ($n = 16$) was 1.9x (range: 1.4–10×) the prescribed amount.

Clinical signs for all patients included diarrhoea (63%), anorexia (56%), vomiting (38%) and nausea (25%). Five patients also experienced lethargy (31%). Hematologic abnormalities for all patients included neutropenia (94%), of which 12/15 (80%) were grade IV, thrombocytopenia (88%) and anaemia (63%). Most patients were hospitalized ($n = 11$) and all but two patients survived the OD.

Conclusion: This is the largest data set to describe the outcomes of accidental anthracycline ODs in canine patients. All patients received supportive care after identifying the OD and death was uncommon. High-grade myelotoxicity was common and may not have been mitigated with the administration of filgrastim. Further evaluation is needed to determine ideal therapeutic guidelines for treatment of a chemotherapy OD.

MULTI-INSTITUTIONAL RETROSPECTIVE STUDY OF CANINE FOOT PAD MELANOMAS: 20 CASES

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Introduction: Melanomas arising from the footpad are a rare clinical entity in dogs. The biologic behaviour of footpad malignant melanoma is not well understood, and these tumours are infrequently described. The primary objective of this study was to evaluate the clinical characteristics of primary canine footpad melanoma in a larger cohort of patients.

Methods: Eligible cases were solicited from the ACVIM Oncology listserv for retrospective review. Included dogs had a cytologic and/or histologic diagnosis of footpad melanoma evaluated by a board-certified clinical or anatomic pathologist. Dogs with cutaneous, oral, digital, subungual or interdigital melanomas were excluded. Complete medical records and follow-up information for at least 8 weeks were required for inclusion.

Results: Twenty dogs from 11 institutions met the inclusion criteria. Most patients (19/20) had surgical resection with (5/19) or without (14/19) preceding pre-operative incisional biopsy. One patient had incisional biopsy alone. At diagnosis, regional lymph node metastasis was observed in four dogs. Eleven dogs received various adjuvant therapies including chemotherapy, radiation therapy, and/or the Oncept melanoma vaccine following surgery. Seven dogs developed subsequent regional and/or distant metastasis. The progression-free interval (PFI) was 101 days (range, 20–960 days). The median survival time (MST) was 240 days (range, 25–479 days). For dogs receiving adjuvant therapy, the MST was 159 days (range, 25–387 days).

Conclusion: Canine footpad melanoma is a rare neoplasm that can exhibit an aggressive behaviour. Adjuvant treatment was not shown to prolong survival. However, further studies are warranted to continue to investigate outcome and significant prognostic factors.

Funding Information: N/A.

READABILITY OF ONCOLOGY DISCHARGE SUMMARIES AT A TERTIARY REFERRAL HOSPITAL

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Introduction: The average American reads at the eighth grade level. The AMA and NIH recommend written health information directed at laypersons target a sixth grade reading level to ensure comprehension by the majority of adults. Veterinary oncologists routinely use written discharge summaries to disseminate complex information to owners. We hypothesized discharge summaries provided to owners of pets newly diagnosed with cancer are written at higher-than-recommended readability levels.
Methods: The readability of client-directed portions of 118 randomly selected discharge summaries written between June 2017 and January 2019 was analysed using two previously validated readability calculators: the Flesch Kincaid Grade Level (FKGL) and the Flesch Reading Ease (FRE) test. Specifically, we examined text related to diagnosis, treatment options, medication(s) and follow-up plan.
Results: The average readability of all summaries determined via the FKGL and FRE were 11.9 (Med 11.9, SD 1.1, range 8.6–15.5; target ≤6) and 43 (Med 42.7, SD 5.9, range 25.5–58.1; target ≥60) respectively. When analysed in their entirety, no discharge summary was written below an 8th grade reading level. Ninety-three percent ($n = 100$) of summaries were difficult to read. There were no significant differences in FKGL or FRE scores among summaries of the four most common tumour types in our sample ($p = .52$ and $p = .67$, respectively.)
Conclusion: Written information targeted at owners of pets newly diagnosed with cancer exceeds the reading comprehension of the average adult. Simpler, shorter summaries incorporating pictures and infographics may improve owner understanding as well as client compliance and patient outcome.
Funding Information: There are no funding sources, or conflicts of interest to disclose.

COMPARISON OF GROSS TUMOUR VOLUME CONTOURS USING COMPUTED TOMOGRAPHY SCANS ALONE VERSUS CO-REGISTERED MAGNETIC RESONANCE FUSION IMAGING GUIDANCE FOR ADAM'S STAGE IV CANINE NASAL TUMOURS

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Introduction: Canine nasal tumours are locally invasive, with stage IV disease including cribriform plate lysis and potentially intracalvarial involvement. Computed tomography (CT) is used to help create conformal plans, as electron density allows for dosimetric prediction in radiation planning. CT alone may not provide the soft tissue contrast needed to discern normal brain from tumour tissue in stage IV patients. Magnetic resonance imaging (MRI) can provide additional soft tissue contrast to aid in contouring for radiation planning. The objective of this study was to assess the use of either CT or CT and MRI for guiding tumour delineation in canine stage IV nasal tumour patients.
Methods: For each patient, gross tumour volume (GTV) was contoured with CT and again with CT-MRI co-registered images at least 1 week later to decrease bias between the two data sets. GTV differences were assessed for normality and a non-parametric Wilcoxon matched pairs test was used to assess for statistical significance.
Results: Forty-three patients with 44 image sets were included. Forty-one image sets included stage IVb disease. The median size of the GTV contoured from the CT was 47.55 cc. The median size of GTV contoured with the CT co-registered to the MRI was 50.65 cc. Median tumour volumes were significantly larger for MRI co-registered image sets ($p = .0047$, 95% CI 0.2–1.6). The median percent difference between the two groups was 1.86%.
Conclusion: MRI may provide more accurate clinical delineation of tumour volumes in stage IV canine nasal tumours, with larger GTV volumes obtained with MRI co-registration.
Funding Information: Not applicable.

A SURVEY OF CANINE CANCER GENOMIC BIOMARKERS BASED ON STRUCTURED LITERATURE REVIEW, META-ANALYSIS AND COMPARATIVE GENOMICS

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Introduction: Canine cancer genomic biomarker data is increasingly abundant, but remains challenging to access, interpret, and integrate into research or clinical practise. Our objective has been to construct a canine cancer genomic biomarker database through systematic review, meta-analysis and harmonization of cancer mutation data from canine and human sources.
Methods: Primary canine papers describing cancer genomics and mutations were retrieved through structured searches from public

sources, filtered for relevance, and curated to capture mutation-level evidence including mutation annotation (harmonized to CanFam 3.1 and Ensembl v99), evidence type and evidence summary. Human oncology drug biomarkers were curated from literature and databases and were 'caninized' using a comparative genomic translator tool. A MySQL relational database was constructed to store and interlink queryable mutation annotations and biomarker associations.

Results: Of thousands of canine papers initially retrieved, ~500 (1983–2021) described diagnostic, prognostic, or predictive relationships of mutations, gene/protein expression and/or epigenetic changes. One hundred forty papers describing mutation-based associations were exhaustively curated, yielding over 1000 biomarker associations from >5000 dogs across 42 tumour types. Inference from human databases and more than 400 human oncology publications yielded >800 'caninized' biomarker associations. These biomarker data are now queryable in a relational biomarker database called Vidium Insight that is routinely updated as new data is published.

Conclusion: Clinically relevant genomic biomarkers are abundant in primary canine literature and inferred human data. Systematic consumption of this growing data within a harmonized relational database forms a foundation empowering the use of genomics in canine cancer research, clinical diagnostics and drug development.

Funding Information: These studies were funded by Vidium Animal Health.

LYMPHOTROPIC NANOPARTICLE MAGNETIC RESONANCE IMAGING FOR DIAGNOSING METASTATIC LYMPH NODES

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Introduction: Lymphotropic nanoparticle magnetic resonance imaging (LNMRI) utilizes ultrasmall paramagnetic iron oxide nanoparticles (USPIOs) for imaging of lymph nodes in patients afflicted with cancer. USPIOs are phagocytized by macrophages and then localized to lymph nodes where they create a susceptibility artefact on gradient echo MRI sequences. Infiltrative cancer cells in lymph nodes cause regions of effacement, which prevents this, allowing for identification of disease. LNMRI has proven to be a highly accurate method of identifying metastatic lymph nodes in human medicine and has recently been investigated for veterinary patients.

Methods: 20 dogs with head and neck tumours were imaged 48 h after injection of USPIOs. Local lymph nodes were evaluated for presence of metastasis then subsequently extirpated. Imaging results were compared to histological analysis and the sensitivity and specificity of LNMRI calculated.

Results: There were a total of 20 dogs and 90 lymph nodes included in this study. Lymph nodes were treated as individual data points. There was a total of 73 lymph nodes that were non-metastatic and 17 metastatic. The overall sensitivity, specificity and accuracy of LNMRI was 69.2%, 93.1% and 86.7% respectively. However, if dogs with mast cell tumours were excluded from analysis the sensitivity, specificity and accuracy rose to 94.1%, 95.9% and 95.6%.

Conclusion: LNMRI is an accurate way to determine the presence of lymph node metastasis in dogs with some types of head and neck tumours. However, LNMRI has only moderate accuracy in dogs with oral or mucocutaneous mast cell tumour.

Funding Information: Morris Animal Foundation Grant, Colorado State University College of Veterinary Medicine and Biomedical Sciences College Research Council Grant and AMAG Pharmaceuticals all provided financial support for this project.

PREDICTING DYNAMIC CLINICAL OUTCOMES OF THE (L-)CHOP CHEMOTHERAPY FOR CANINE LYMPHOMA PATIENTS USING AN ARTIFICIAL INTELLIGENCE MODEL

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Introduction: Predicting clinical outcomes and survival of cancer patients treated by given chemotherapy can assist in choosing the course of treatment. We developed a methodology for predicting clinical outcome and progression-free survival (PFS) of canine lymphoma patients treated by (L-)CHOP chemotherapy.

Methods: We collected live cancer cells from fresh FNA taken from affected lymph nodes, as well as the response and prognosis of 242 canine lymphoma patients treated by (L)-CHOP for at least 4 weeks. We used three types of data from ex vivo chemosensitivity, flow cytometry and bloodwork to train a machine-learning model that predicts the probability of achieving complete remission at the 4th, 8th, or 12th week of the protocol. The same set of data was also used to predict PFS by utilizing the Cox proportional hazards model.

Results: The predictive accuracy of machine learning models was as high as 80.4%, 89.1% or 82.7% when predicting the clinical outcome after 4th, 8th or 12th week. The performance of the Cox hazards model for predicting PFS was also high, featuring the C-statistic of 0.850. The stratification of the patients based on both the subtype (B- vs. T-cell) and the Cox hazards model outperformed the one based on only the subtype when analysing PFS.

Conclusion: The results demonstrate substantial enhancement in the predictive accuracy by incorporating a greater variety of data. They also highlight superior performance in predicting survival compared to the conventional stratification method. We believe that the proposed

methodology can contribute to improving and personalizing the care of canine lymphoma patients.

Funding Information: This work was self-supported by ImpriMed, Inc.

ZOLEDRONIC ACID AND COARSELY FRACTIONATED EXTERNAL BEAM RADIOTHERAPY: EFFECTIVE LIMB-SPARING THERAPY FOR CANINE OSTEOSARCOMA

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Introduction: Zoledronic acid (ZA) is a third-generation bisphosphonate with high affinity for areas of bone resorption. Preclinical studies have investigated its role as an anti-neoplastic agent, both independently and synergistically, with radiation therapy (RT). ZA + RT act synergistically in numerous human neoplastic cell lines. The exact mechanism of ZA's radio sensitization has not been fully elucidated. It may also have immunomodulatory effects on the tumour microenvironment, including tumour-associated macrophages.

Methods: We investigated cell viability and apoptosis in canine osteosarcoma (OS) cells treated with ZA + RT: 0, 2, 4 and 8 Gy of RT and 10-fold increases of ZA. Second, we evaluated cell cycle arrest at 0, 4, 24 and 48 h post ZA, to decipher an ideal neo-adjuvant timepoint. Finally, we treated 20 dogs with naturally occurring OS with 0.1 mg/kg ZA IV 24 h prior to receiving 8 Gy of RT (once weekly fraction x 4 weeks).

Results: Apoptosis was significantly increased and viability decreased in ZA + RT treated canine OS cells. Preliminary data showed ZA treatment shifts cells from G1 to S phase. Twenty dogs were prospectively treated with ZA + RT and had MST of 254 days; dogs receiving adjuvant carboplatin ($n = 11$, MST 444 days) had significantly longer MST compared to dogs that did not ($n = 9$, MST 211 days, $p = .02$). 2/20 dogs (10%) developed pathologic fracture.

Conclusion: With clinically acceptable survival times noted in our canine OS patients and a low fracture rate, ZA + RT is an appropriate treatment for non-surgical candidates. Future studies should evaluate the immune role ZA + RT play in both human and canine OS.

Funding Information: Intramural funds.

SINGLE CELL T CELL RECEPTOR REPERTOIRE PROFILING FOR DOGS

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Introduction: Precisely tracking tumour-specific immune responses in clinical trials requires reagents to perform species-specific single cell T cell receptor sequencing (scTCRseq). This technology defines clones of T cells reacting to immune interventions and can identify the specific target epitope. Gene expression data give insights into the activity and polarization of the T cell.

Methods: Samples from two responding dogs in a trial of an autologous deglycosylated melanoma vaccine were selected to demonstrate applicability of scTCRseq in cancer immunotherapy. A single-cell suspension of cryopreserved peripheral blood mononuclear cells (PBMC) was prepared for 10× single cell sequencing. Libraries were amplified using a custom-designed nested PCR of the alpha/beta V(D)J region. This enriched product was added to the gene [removed] GEX) library and scTCRseq performed on a NovaSeq 6000.

Results: 1850–2172 estimated V(D)J-expressing cells yielded 87–103.7 million reads with 73.8%–75.8% mapped to a V(D)J gene (beta/alpha chains ratio 1.5:1). 43 TRAJ, 29 TRAV, 12 TRBJ and 22 TRBV genes were observed representing 72.9%, 51.8%, 100% and 62.9% of all known V and J segments respectively. GEX enriched libraries successfully defined large clusters of CD8+ and CD4+ T cells consistent with previously reported results in human PBMC samples. Both dogs exhibit a small number of highly abundant T cell clones suggesting the presence of an anti-tumour T cell population.

Conclusion: The developed reagents successfully generated scTCRseq data that allowed the T cell repertoire to be surveyed in dogs responding to anti-tumour immunotherapy. These reagents will allow longitudinal tracking of anti-tumour T cell responses in canine cancer immunotherapy trials.

Funding Information: Funded by the Siteman Investment Program jointly by Siteman Cancer Center and Ellis Fischel Cancer Center.

PRECLINICAL DEVELOPMENT OF BISPECIFIC CAR T CELLS FOR CANINE B CELL MALIGNANCIES

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Introduction: Chimeric antigen receptor (CAR)-T cells targeting CD19 have revolutionized the treatment of human B cell malignancies. However, post-treatment loss of CD19 has emerged as an adaptive

mechanism of resistance. Comparative trials at the University of Pennsylvania are investigating the activity of canine CAR-T cells targeting either CD19 or CD20 in canine B cell lymphoma patients. Similarly, expansion of CD20-negative B cells was observed in dogs treated with anti-CD20 CAR-T cells. Bispecific CAR-T cells that simultaneously target two antigens to mitigate antigen escape have encouraging efficacy profiles in human patients. Thus, we designed a dual-targeting CAR specific for canine B cell antigens.

Methods: A second-generation canine CAR construct targeting both CD19 and CD20 was cloned into the MSGV1 backbone. Chimeric retrovirus encoding RD114 and VSV-G envelope proteins and the CAR construct was made using GP2-293 cells. Jurkat NFAT-GFP reporter cells and primary T cells from dogs diagnosed with high-grade B cell lymphoma were retrovirally transduced. Antigen specific reactivity was assessed against cell lines with variable CD19 and CD20 expression patterns.

Results: The CD19-CD20 CAR was efficiently transduced, with stable expression in both Jurkat and primary canine T cells. Jurkat reporters revealed dual-specific CAR signalling after co-culture with CD19+ and/or CD20+ target cells. Primary canine CAR-T cells expanded in vitro and exhibited CAR-specific proliferation and cytotoxicity against CD19+ CD20+ target cells.

Conclusion: Preclinical manufacture of functional, bispecific anti-CD19 anti-CD20 canine CAR-T cells is feasible. A clinical trial recruiting dogs diagnosed with B cell cancer will assess safety and efficacy in vivo.

Funding Information: Shuman Translational Research Fellowship (MJA); NCI K08CA252619 (MJA); PetCo Love (NJM).

CHARACTERIZATION OF A FULLY CANINE ANTI-CANINE CTLA4 ANTIBODY FOR CHECKPOINT INHIBITION IN CANINE CANCER

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Introduction: The anti-CTLA4 checkpoint inhibitor ipilimumab, has revolutionized cancer patient treatment by promoting effector T cell function and eliminating intratumoral regulatory T cells. However, only a subset of patients respond clinically. Pet dogs with spontaneous tumours represent a valuable patient population to investigate predictive biomarkers and rational therapeutic checkpoint inhibitor combinations. Furthermore, dogs with immunogenic tumours might benefit clinically from CTLA4 inhibition. Here, we generate and validate a fully canine anti-CTLA4 monoclonal antibody for translational research and clinical veterinary use.

Methods: Fully canine single chain variable fragments (scFv) that bind canine CTLA4 (cCTLA4) were isolated from a comprehensive canine scFv phage display library. scFv were evaluated for cCTLA4 binding by flow cytometry and surface plasmon resonance and by ELISA for their ability to inhibit cCTLA4:CD80/CD86 interaction. The effects of cCTLA4 blockade on mitogen-activated canine T cell function were evaluated in vitro and biodistribution studies were performed in mice.

Results: A selected fully canine anti-CTLA4 clone specifically bound cCTLA4 with subnanomolar binding affinity, inhibited cCTLA4 binding to CD80/CD86 and promoted T cell proliferation and effector function. The antibody exhibited linear kinetics in vivo in mice and produced no short-term clinical or haematological adverse effects.

Conclusion: This work paves the way for in vivo analysis of the first fully canine, anti-canine CTLA4 antibody to promote anti-tumour immunity in dogs with immune responsive cancers and provides an important comparative tool to investigate correlative biomarkers of response and mechanisms of resistance to CTLA4 checkpoint inhibition.

Funding Information: V Foundation for Cancer Research.
NCI/NIH/SBIR Contract 75N91018C000042.

THE TOLERABILITY AND ANTICANCER IMMUNE ACTIVATING PROPERTIES OF INTRATUMORAL CANINIZED COLLAGEN-ANCHORED INTERLEUKINS-2 AND -12 FOR INDUCING LOCAL AND ABSCOPAL EFFECTS IN DOGS WITH MEASURABLE MALIGNANT MELANOMA

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Introduction: The field of immune-oncology (IO) has exploded over the past decade with scores of innovative immunotherapeutic strategies. Historically, cytokines have held promise for amplifying adaptive immune responses against immunogenic cancers; yet despite their documented activity, the systemic administration of pro-inflammatory cytokines, that is, IL2/IL12 has been hampered by severe adverse or even fatal on-tumour, off-target toxicities. To reap benefits of cytokine strategies, yet minimize toxicities, protein-engineering efforts have focused on intratumoral cytokine retention innovations capable of promoting intense and sustained localized immune activation sufficient for local tumour control and exertion of potent abscopal effects against metastases. In this study, we have generated caninized collagen-anchored IL2/IL12 (CaCol-IL2/12) as an innovative intratumoral delivery strategy, and evaluated tolerability, pharmacodynamics, immune-activation and therapeutic effects in healthy beagle and dogs with measurable malignant melanoma (MMM).

Methods: Beagle dogs were included for MTD assessment of intradermal CaCol-IL2/12. Dogs with MMM were recruited (3 + 3 design) to be treated with a single fraction of radiation (9gy), followed with bimonthly CaCol-IL2/IL12 intratumoral injections with hematologic, biochemical, immunologic and radiologic reevaluations.

Results: Conservative MTD of CaCol-IL2/IL12 in beagles was 0.2 mg/kgIL2/0.022 mg/kgIL12. Six dogs with MMM were treated at 1× and 2× MTD. Treatments were well tolerated with dogs developing transient fever and self-limiting localized facial edema. Strong local cytoreduction was achieved in four of six patients, and evidence for abscopal activities on regional metastases was observed too.

Conclusion: CaCol-IL2/IL12 is a tolerable and active immunotherapeutic strategy that holds promise for reaping the benefits of cytokine manipulation yet minimizing systemic toxicities.

Funding Information: None.

IDENTIFICATION OF SOMATIC MUTATIONS IN CANINE TUMOURS USING AN EFFECTIVE GERMLINE-SOMATIC DISCRIMINATION PIPELINE WITHOUT MATCHING NORMALS: PRECISION MEDICINE APPLICATION

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Introduction: Accurate somatic mutation identification in NGS data is a critical step for a genomics approach since it can identify driver mutations and support clinical decision-making through target therapy. Different from germline variants, somatic mutations are the most common cause of cancer.

Methods: In this study, we described an effective pipeline to differentiate germline and somatic mutation using tumour-only next-generation sequencing (NGS). In the first step, 2320 total canine germline mutations were filtered out for somatic evaluation by comparison with previous publications and genomic databases. After that, mutations identified in more than five dogs with variant allele frequency (VAF) distribution centred at 0.5 (heterozygous germline) and 1.0 (homozygous germline) were filtered out (494 germline mutations). For mutations identified in less than five dogs, the protein sequence alignment between human and dog were compared. During this process, 415 unclassifiable mutations were excluded.

Results: A total of 915 somatic mutations were identified using this filtering system. The VAF distribution of putative germline variants filtered out in this pipeline resembles known germline mutation, but not the TP53 and PIK3CA variants, which are somatic, indicating this is a valid method.

Conclusion: This is an effective and more affordable germline-somatic mutation discrimination methodology compared to traditional correction system that uses a matched normal sample from a health tissue of the same dog. Using our pipeline, we discovered canine mutations in 50 well-established oncogenes and tumour suppressors, and compared them to those reported in human cancers.

ESTABLISHMENT OF TOLERABILITY AND PHARMACOKINETIC PARAMETERS FOR ETHOS-PUSH (ETHOS-PRECISION MEDICINE UMBRELLA STUDY FOR HEMANGIOSARCOMA) ANTICANCER AGENTS IN CANCER-BEARING DOGS

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Introduction: Canine hemangiosarcoma (HSA) is an aggressive cancer that is poorly responsive to chemotherapy. Ethos-PUSH seeks to identify novel treatments for dogs with splenic HSA that improve clinical outcomes, molecular biomarkers of prognosis, and biomarker-drug matches associated with exceptional drug response. A necessary step in successful oncology drug development includes drug selection criteria and studies of drug tolerability in tumour-bearing patients.

Methods: Criteria for drug selection in this Umbrella study design included: biological rationale from hemangiosarcoma studies, novel mechanism of action, or hints of activity in canine HSA or human angiosarcoma. Walk-in studies were performed in cancer-bearing dogs to establish the safety/tolerability and pharmacokinetic (PK) parameters for Oral Paclitaxel (Oraxol), Sorafenib, and a novel, oil-based formulation of Rapamycin.

Results: Paclitaxel has demonstrated broad activity against canine and human cancers (including angiosarcoma). Using a rolling-6 tolerability study design, we established a clinically relevant dose/schedule of Oraxol (90 mg/m^2 on three consecutive days weekly) in pet dogs with cancer. Sorafenib is a dual inhibitor of VEGF and RAF/MAPK and has unique value among multi-targeting TKIs as it may circumvent the problem of evasive resistance associated with VEGF inhibition alone. We defined the tolerability and PK-based dosing of Sorafenib (3 mg/kg daily) in tumour-bearing dogs. Aberrant mTOR signalling is a biological feature of HSA. We report on the tolerability and desired PK of an oil-based formulation of Rapamycin designed to overcome challenges associated with poor oral bioavailability.

Conclusion: These data establish the tolerability, PK parameters and dosing schemes for Ethos-PUSH anticancer agents in cancer-bearing dogs.

Funding Information: Ethos Discovery, San Diego, CA.

TWO PROSPECTIVE TRIALS DEMONSTRATE LOWER RISK FOR MALIGNANCY IN CANINE HEMOPERITONEUM SECONDARY TO RUPTURED SPLENIC TUMOURS

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Introduction: A common cause of spontaneous hemoperitoneum in dogs is the rupture of splenic masses. Previous retrospective studies have reported the cause of spontaneous hemoperitoneum to be due to benign (curable) lesions in as low as 4% of cases. The collective results of two sequentially launched prospective studies now suggest a new narrative on the prevalence of benign tumours and perioperative outcomes in older large breed dogs with hemoperitoneum from ruptured splenic masses.

Methods: There were 40 dogs recruited in the first study and 60 dogs in the second study. All dogs underwent pre-surgical staging, splenectomy, and were followed until discharge. Biospecimens were collected for genomic analysis as part of a parallel study. All other perioperative care was at the discretion of the attending clinician.

Results: Both cohorts predominately consisted of older large breed dogs. 35/100 (35%) of dogs had benign splenic tumours that were cured with surgery alone 93/100 (93%) survived to discharge respectively.

Conclusion: These prospective cohort data yield distinctive and more optimistic perspectives for dogs with hemoperitoneum due to splenic rupture than past retrospective studies. These data now allow veterinarians to consider this new optimism during discussions about splenectomy for dogs with hemoperitoneum. In parallel, we are using genomic data from this cohort to identify improved therapeutic options for dogs with hemangiosarcoma utilizing a precision medicine approach. Similar efforts were responsible for the transformation of childhood leukaemia from a commonly fatal cancer to one that is now commonly cured.

Funding Information: All funding was provided by Ethos Discovery (501c3).

THE SHINE ON PROGRAM FOR EARLY DETECTION AND PREVENTION OF CANINE HEMANGIOSARCOMA

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Introduction: Hemangiosarcoma is a common, fatal cancer of dogs that grows insidiously without pain or obvious symptomatology, so diagnosis usually happens late in the course of disease or after death. A precision medicine approach of early detection and prevention could significantly reduce the burden of hemangiosarcoma in companion dogs.

Methods: The shine on suspicion (SOS) test uses flow cytometry to detect hemangiosarcoma-associated cells in circulation and machine learning for risk assignment. 10× Genomics single cell RNA sequencing was used to define the ontogeny of hemangiosarcoma-associated cells. Ninety-seven samples from dogs with known conditions constituted the training set and 209 samples from clinically healthy dogs older than 6 years of age constituted the validation set for machine learning. A single cycle of eBAT was used for prevention.

Results: The SOS test achieved 89% sensitivity and 95% specificity for hemangiosarcoma detection. Our results suggest that the test can be used to assign dogs into risk categories for hemangiosarcoma by detecting circulating cells up to 24 months before gross disease develops. While follow-up will continue through the lifetime of every dog in the study, early results suggest that eBAT prevention can diminish risk of hemangiosarcoma based on repeated SOS testing.

Conclusion: The SOS test accurately detects hemangiosarcoma-associated cells in blood from dogs with active disease and can be used to categorize the risk of healthy dogs to develop hemangiosarcoma. The test has acceptable performance metrics, and combined with eBAT, can provide an actionable platform for early detection and strategic prevention of canine hemangiosarcoma.

Funding Information: This work was supported by grants CHF-02234-MOU and CHF-02806-MOU from the AKC Canine Health Foundation, by the Alvin and June Perlman Chair in Animal Oncology, and by generous individual donations to the Animal Cancer Care and Research Program of the University of Minnesota.

SEQUENCING THE EPIGENOME OF SPLENIC TUMOURS MAY UNCOVER NEW INSIGHT INTO THE BIOLOGY AND THERAPY OF CANINE SPLENIC HEMANGIOSARCOMA

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Introduction: Epigenetic dysregulation of gene expression in cancer provides a valuable perspective to explain cancer biology and predict novel therapeutic opportunities. Conventional genomic techniques have failed to identify recurrent genetic alterations in complex veterinary cancers, like hemangiosarcoma. The Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq) profiles regions of the genome accessible to transcription factor binding and gene transcription providing a snapshot of epigenetic alterations in cancer. The goal of this pilot study is to profile epigentic changes in splenic hemangiosarcoma and benign splenic tumours using ATAC-seq.

Methods: Samples were collected via an ongoing 400 dog prospective clinical trial of canine splenic hemangiosarcoma (Ethos-PUSH). ATAC-seq has been performed on >2 distinct tumour regions with matched normal splenic samples from seven tumours (four hemangiosarcoma, two nodular hyperplasia/hematomas, one splenic marginal zone lymphoma). Histopathology of all tumour and normal samples was performed by a single pathologist to confirm diagnosis and tumour content.

Results: Benign hematomas/nodular hyperplasia and hemangiosarcoma share open chromatin patterns, while marginal zone lymphoma has a distinct chromatin pattern. Furthermore, matched normal samples share open chromatin patterns with adjacent tumour samples despite lacking evidence of neoplasia on histopathology.

Conclusion: Preliminary results suggest dogs who develop benign hematoma/nodular hyperplasia and hemangiosarcoma have a shared spleen-wide epigenetic signature. This signature suggests a common epigentic event that fosters neoplastic transformation of endothelial precursor cells in benign and malignant neoplasms and implicates epigenetic targeting drugs could be useful in modulating tumour microenvironment and treating canine splenic hemangiosarcoma.

Funding Information: Ethos Veterinary Health.
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PROPRANOLOL REDUCES HEMANGIOSARCOMA CELL VIABILITY BY DISRUPTING LIPID HOMEOSTASIS AND INDUCING ER STRESS

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Introduction: Canine hemangiosarcoma is an aggressive and lethal tumour in dogs that closely mimics the clinical presentation of human angiosarcoma. The beta adrenergic receptor antagonist, propranolol, has recently been shown to promote tumour regression and increase the overall survival of angiosarcoma patients. Preclinical models suggest propranolol may also be effective against hemangiosarcoma. Our objective was to determine if pathways vital to hemangiosarcoma cell survival are vulnerable to propranolol inhibition.

Methods: RNA-sequencing (RNA-seq) was used to identify altered transcriptional programs. Fluorescent-based assays in combination with flow cytometry and confocal microscopy were used to confirm inhibition of autophagic flux, endocytosis and metabolic pathways in hemangiosarcoma cell lines. Immunoblotting and qRT-PCR were used to confirm activation of the lipid synthesis and endoplasmic reticulum (ER) stress pathways.

Results: Our data suggest that propranolol prevents hemangiosarcoma cells from obtaining the essential lipid building blocks needed for cell proliferation and viability. Propranolol blocked endocytosis and autophagic flux, preventing the uptake and processing of exogenous cholesterol and fatty acids. In response, cells rapidly increased the expression of genes and proteins involved in fatty acid and cholesterol synthesis via activation of the transcription factor SREBP-1. In addition, propranolol induced the ER stress pathways; we associated the extent of activation of these pathways in hemangiosarcoma cell lines with propranolol sensitivity.

Conclusion: We conclude that propranolol inhibits hemangiosarcoma cell proliferation and induces cell death by disrupting lipid homeostasis and inducing ER stress. Targeting these pathways in combination with propranolol may provide new opportunities for strategic intervention.

Funding Information: This work was supported by grants D17CA-059 and D18CA-017 from Morris Animal Foundation, the Sarcoma Foundation of America (Dr. Richard and Valerie Aronsohn Memorial Research Award), and P30 CA077598 from the National Institutes of Health.

A RETROSPECTIVE ANALYSIS EVALUATING THE OUTCOME OF DOGS WITH ORAL MALIGNANT MELANOMA TREATED WITH 6 GY X 6 RADIOTHERAPY PROTOCOL

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Introduction: Canine oral malignant melanoma (OMM) is a locally aggressive oral tumour with high risk of locoregional and distant metastasis. With multimodal therapy, most dogs will succumb to OMM within 1–2 years. Locoregional therapy typically includes surgery, radiation therapy (RT), or a combination thereof. Current practise patterns favour the use of hypofractionated RT. One commonly used protocol is 6 Gy x 6 fractions; however, this protocol is not well-described. Thus, the objective of this tri-institutional retrospective study was to describe oncologic outcomes.

Methods: Dogs were included in the analysis if they underwent 6 Gy x 6 irradiation to manage micro- or macroscopic OMM. Progression-free and overall survival times were characterized using Kaplan–Meier statistics and Cox regression modelling. Univariate analyses were performed to assess the prognostic value of various factors including: dose intensity (weekly vs. biweekly; $n = 57$ and 44, respectively), treatment planning type (manual vs. computerized; $n = 44$ and 48, respectively, 9 unknown), treatment of micro- or macroscopic disease ($n = 36$ and 65, respectively) and tumour stage.

Results: For the entire group ($n = 101$ dogs), the median progression-free survival and overall survival times were 171 and 232 days, respectively. Based upon univariate analysis, variables that were statistically significantly ($p < .05$) included: presence of macroscopic disease ($p = <.0001$), bony lysis ($p = .0253$), advanced tumour stage ($p = .0079$) and metastasis at diagnosis ($p = .0002$).

Conclusion: The final statistical model (Cox multivariable regression) will be built upon completion of data collection.

ASSESSMENT OF CANNABINOID RECEPTOR EXPRESSION AND ANTI-NEOPLASTIC EFFECTS OF CANNABIDIOL (CBD) IN CANINE UROTHELIAL CARCINOMA CELLS

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Introduction: Despite existing combination therapies, local and systemic control of canine urothelial carcinoma (UC) remains challenging. Increasingly, cannabidiol (CBD) has been evaluated for anti-neoplastic effects in vitro and in vivo, yielding encouraging results. However, the mechanisms through which these occur remain incompletely understood. Although these effects have been studied in various human malignancies, including urogenital tumours, there is a lack of such information available for veterinary patients. This study aimed to evaluate expression of various cannabinoid receptors in canine UC cell lines, examine the anti-neoplastic effects of CBD and investigate their underlying mechanisms.

Methods: Three canine UC cell lines and one immortalized canine kidney epithelial cell line were evaluated. Expression of cannabinoid receptors CB1, CB2, PPAR-gamma, GPR55 and TRPV1 was evaluated via western blotting. Crystal violet colorimetric assays were used to establish IC50 concentrations for CBD and assess the effects of known cannabinoid receptor antagonists on cell viability. Flow cytometry was used to measure apoptosis and autophagy to characterize the mechanisms through which CBD induces cytotoxicity.

Results: The expression of cannabinoid receptors was detected in all cell lines. Treatment with CBD resulted in dose dependent reduction of cell viability in all cell lines; however, apoptosis and autophagy were not significantly increased compared to vehicle control at 8 and 24 h respectively. Treatment with receptor antagonists did not alter cell viability in comparison to treatment with CBD alone.

Conclusion: Further investigation into possible underlying mechanisms of CBD as a novel therapeutic agent is necessary before its role in improving patient outcomes can be established.

Funding Information: Funding for this project was provided by Greywolf Animal Health and Ontario Veterinary College Pet Trust.

CLINICAL OUTCOME IN DOGS WITH APPENDICULAR OSTEOSARCOMA TREATED WITH PALLIATIVE RADIATION THERAPY +/− BISPHOSPHONATES

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Introduction: Hypofractionated radiation therapy (RT) and bisphosphonates are commonly utilized for palliative intent treatment of canine osteosarcoma (OSA). Consensus has not been reached as to whether these treatments should be administered concurrently. The primary objective of this study was to evaluate outcome in dogs treated with hypofractionated RT, with and without the addition of bisphosphonates. A secondary objective was to identify prognostic factors in this population.

Methods: Dogs with presumed and confirmed OSA of the appendicular limb treated with daily hypofractionated RT (8 Gy x 2 fractions) at the Flint Animal Cancer Centre between 2010 and 2019 were evaluated retrospectively. Clinical data were abstracted from the medical records, and adjuvant therapies were noted. Outcome was assessed using medical records and electronic follow-up.

Results: 165 dogs were included. Sixty-eight dogs received bisphosphonates as part of their palliative intent treatment. Median survival time from the first RT treatment to death was not significantly different between groups ($p = .758$). Only age (>/= 9 years) was found to be prognostic in this population ($p = .031$). Factors not associated with survival time included bisphosphonate drug type, timing of bisphosphonate administration, tumour location, weight, breed, sex, time from diagnosis to treatment, concurrent administration of chemotherapy and amputation.

Conclusion: This study suggests no difference in outcome for dogs treated with and without bisphosphonates in addition to hypofractionated RT. Prospective studies are needed to determine if the addition of bisphosphonates to hypofractionated RT leads to an improved quality of life in dogs undergoing palliative intent treatment for OSA.

COMPLICATIONS AND SHORT-TERM OUTCOMES ASSOCIATED WITH BILATERAL MULTIPLE CERVICAL LYMPH NODE EXTIRPATION FOR MAXILLOFACIAL TUMOURS IN DOGS

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Introduction: Due to the extensive lymphatic crossover of the head and neck, bilateral cervical lymphadenectomy is recommended when staging oral tumours. This technique is frequently performed through a ventral cervical non-selective regional lymphadenectomy. The purpose of this study was to report complications and short-term outcomes in patients undergoing cervical regional lymphadenectomy for staging in dogs with maxillofacial tumours.
Methods: Medical records were reviewed from five academic referral hospitals from 2012 to 2020 with at least 1 ACVS Fellow of Surgical Oncology. All supervising surgeons had performed >5 cervical regional lymphadenectomy procedures. Patients were included if they underwent a standard ventral cervical approach with bilateral removal of at least 1 mandibular and medial retropharyngeal lymph node per side through a single ventral cervical incision. A minimum follow-up period of 2 weeks was required for inclusion.
Results: Seventy-five dogs met the inclusion criteria. Primary tumour location included mandible (28), maxilla (27), lip (15), tongue (4) and unknown (1). A median of 6 (range 4–8) lymph nodes were removed per dog. At the time of discharge, facial swelling was documented in 35 dogs (46.7%) and cervical swelling in 28 (37.3%). At suture removal, one dog had persistent facial swelling and a seroma was present in 13. Three dogs (4%) developed a cervical abscess that required debridement.
Conclusion: Head swelling frequently occurs patients undergoing regional cervical lymphadenectomy, although aetiology may be multifactorial. This swelling appeared to be self-limiting and resolved by 14 days postoperatively in most cases. Seroma formation was common and may persist beyond suture removal.

EARLY FAILURE OF CHOP PROTOCOL INDICATES POOR RESPONSE TO RESCUE PROTOCOL

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Introduction: Dogs with lymphoma that fail CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) chemotherapy before completion of their protocol are commonly thought to have a poor long-term outcome; however, no previous studies have evaluated the effect of early CHOP relapse on progression free interval (PFI) or overall survival time (OST) for patients undergoing rescue chemotherapy. The aim of this study was to determine if dogs with multicentric lymphoma that fail CHOP prior to completion have a worse outcome with rescue therapy than dogs that complete CHOP.
Methods: Data were collected from six previous retrospective or prospective studies in 193 dogs with multicentric lymphoma that received CHOP-based chemotherapy then received either lomustine (CCNU), L-asparaginase and prednisone or rabacfosadine (Tanovea-CA1), with or without prednisone or L-asparaginase.
Results: Length of CHOP PFI (progression during versus after completion of CHOP) was significantly associated with PFI and post-relapse OST for both rescue protocols. Patients achieving a complete response to CHOP had a significantly longer PFI for both rescue protocols. Immunophenotype (B vs. T cell) was not significantly associated with response, PFI or OST for L-asparaginase/lomustine but was significantly associated with PFI for rabacfosadine.
Conclusion: Dogs with multicentric lymphoma that fail a CHOP protocol prior to completion are likely to have shorter PFI and OST with rescue therapy. Immunophenotype does not significantly affect outcome with L-asparaginase/lomustine but is associated with PFI for rabacfosadine.
Funding Information: This work was supported by VetDC and Elanco (FDA grant# 1R01 FD006323-01).

EARLY FEASIBILITY TRIAL OF HIGH-FREQUENCY IRREVERSIBLE ELECTROPORATION (H-FIRE) FOR THE TREATMENT OF CANINE HEPATOCELLULAR CARCINOMA

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Introduction: High-frequency irreversible electroporation (H-FIRE) is a non-thermal ablative technique that utilizes short, intense, bipolar electrical pulses to create permanent nanopores in cellular membranes resulting in cell death. We hypothesize that treatment of canine hepatocellular carcinoma (HCC) with H-FIRE results in preferential neoplastic cell death. Our objective is to characterize the response and lethal threshold of HCC and non-neoplastic liver to H-FIRE, and the tumour microenvironment response to the treatment.

Methods: Dogs with HCC were recruited and staged with triple-phase CT. 3D modelling for treatment planning was performed using finite 3D element analysis and H-FIRE was delivered with an open approach through 2–5–2 us bursts to the tumour and non-neoplastic liver. Tumour was excised and patients were recovered in ICU. Abdominal CT was performed at 24 h, 4 days and 1 month post-treatment to monitor the non-neoplastic liver ablation. Histopathology and IHC for Iba-1, CD3, CD79a and FOXP3 was performed to characterize tumour ablation and potential immune cell infiltrates. Tumour microenvironment changes were evaluated using the Nanostring canine immuno-oncology panel.

Results: Three dogs have been treated with no dogs experiencing complications associated with H-FIRE. Histopathology of treated HCC demonstrated ablation characterized by focal haemorrhage and hepatocyte degeneration. Follow-up CT on the non-neoplastic liver treated with H-FIRE suggests that ablation volumes for non-neoplastic liver are smaller than those of HCC, under the identical pulse parameters. H-FIRE results in intratumoral increase in immune cell infiltrate and activation of phagocytic, cytotoxic T-cell and NK-cell activity.

Conclusion: Our findings suggest H-FIRE preferentially targets neoplastic cells.

Funding Information: Veterinary Cancer Society Theilen Resident Research Grant.
Grayton Frielander Memorial Fund.

ENRICHMENT TOOLS TO BETTER UNDERSTAND THE DIFFERENT TYPES OF CIRCULATING NUCLEOSOMES AND THEIR ASSOCIATED GENOME PATTERNS IN THE PLASMA OF DOGS WITH LYMPHOMA

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Introduction: Nucleosomes contain DNA wrapped around a core histone octamer. Following cell death nucleosomes are released into circulation and thus diseases with high cellular turnover rates (e.g., cancer) can have increased nucleosomes in plasma. Interestingly, cancer-derived nucleosomes have shorter DNA (147 bp) than nucleosomes derived from non-cancerous cellular turnover, thus enabling isolation and specific analysis of cancer-derived nucleosomes.

The aim of this study was to isolate and sequence cancer-derived nucleosomes from dogs diagnosed with lymphoma thereby reducing the complexity of liquid biopsy and enabling the identification of biomarkers of clinical significance.

Methods: Plasma samples from healthy dogs and dogs with lymphoma were collected with owner consent and a proprietary enrichment method (Nu.Q Capture) was used to separate short (cancer-derived) from long (non-cancer-derived) nucleosomes. The resulting DNA was sequenced and aligned to the CanFam4 genome. Fragment length and copy number estimates were calculated.

Results: Following enrichment we found that, as expected, the unbound supernatant contained exclusively mono-nucleosomes whereas the captured fraction contained mono, di and tri nucleosomes. Furthermore, we found evidence of increased proportion of shorter nucleosomes (147 bp DNA) in dogs with lymphoma, consistent with findings in humans.

Conclusion: Circulating nucleosomes can be enriched from canine lymphoma patients based on DNA size, enabling a less complex substrate for identifying cancer-associated genetic aberrations. Further studies are ongoing to determine the scope of DNA alterations that can be identified and what role these differences play in treatment response, minimal residual disease status, progression free survival and prognosis.

Funding Information: Funding provided by Volition Veterinary Diagnostics.

IMMUNOPHENOTYPIC ABERRANCIES DO NOT SEEM TO REPRESENT A NEGATIVE PROGNOSTIC FACTOR IN HIGH-GRADE CANINE T-CELL LYMPHOMAS

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Introduction: Immunophenotypic aberrancies can be detected by flow-cytometry (FC), however, their prognostic significance in high-grade canine lymphoma remains unknown.

The aim of this retrospective study was to evaluate if the presence of immunophenotypic aberrancies carries a worse prognosis in dogs with high-grade T-cell lymphoma (TCL) treated with lomustine-based protocols.

Methods: Medical records from six institutions between 2010 and 2021 were reviewed. Dogs with a diagnosis of high-grade TCL through cytology and FC (that included antibodies for CD45, CD3, CD5, CD4, CD8, CD21, CD79a, MHCII and CD34), and treated with lomustine-based protocols were included.

Information regarding stage, sub-stage, treatment type, response to treatment, progression-free survival (PFS), and median survival time (MST) were recorded. Immunophenotypes were considered aberrant when showing qualitative alterations in receptor expression: loss or diminished expression of expected antigens and/or co-expression of markers of different cell lineage.

Results: Fifty-five dogs met the inclusion criteria and were divided into two groups: aberrant group (AG) including 28 dogs, and non-aberrant group (NAG) including 27 dogs. Groups were considered homogeneous in terms of stage, sub-stage and overall response to treatment (with AG having 89.2% of responders and NAG 88.8% of responders). Median PFS time was 126 days for AG and 190 days for NAG ($p = .226$). MST was 165 and 275 days for AG and NAG, respectively ($p = .205$). No statistical differences were found in MST and PFS between dogs with aberrant and non-aberrant high-grade TCL.

Conclusion: Immunophenotypic aberrancies do not seem to represent a prognostic factor in our study population.

Funding Information: None.

PILOT STUDY OF PARTIAL ABLATION WITH MECHANICAL HIGH-INTENSITY FOCUSED ULTRASOUND (HISTOTRIPSY) IN DOGS WITH SPONTANEOUSLY OCCURRING SOFT TISSUE SARCOMAS

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Introduction: Histotripsy is a non-thermal and non-invasive high-intensity focused ultrasound ablative technique that causes mechanical fragmentation of tissue resulting in liquefied cellular debris with histologically clear demarcated boundaries between treated tissue and non-treated tissue. Histotripsy has never been evaluated in a spontaneous cancer model. Our objective was to evaluate the safety and feasibility of histotripsy to achieve tumour ablation in dogs with soft tissue sarcoma (STS).

Methods: Dogs diagnosed with STS were recruited. CT of the chest, abdomen, and the tumour was performed for staging and treatment-planning, and pre-treatment biopsy was obtained. Safety was monitored with exams, owner reports and CBC/serum biochemistry. Partial tumour ablation was performed using a prototype Histotripsy system. Anatomical ablation characteristics were evaluated with contrast CT at 1- and 4-days post-treatment, with tumour resection 4-days post-treatment. Tumour ablation effectiveness was evaluated with H&E.

Results: Ten dogs were recruited and treated. Tumour histologies included three grade III STS, 4 grade II STS, 2 grade I STS and 1 malignant mesenchymoma. Currently, seven dogs are alive, two dogs were euthanized from recurrence or suspected metastasis and one dog was lost to follow-up. There were no changes in bloodwork values. The mean planned ablated volume was 8.41 ± 5.01 cm^3. The mean duration of treatment was 30.43 ± 12.67 min. Histotripsy-related complications were generally self-limiting and included various degrees of cutaneous injury. Post-treatment histopathology indicated complete ablation of targeted tumour with no intact cells identified.

Conclusion: Histotripsy can achieve safe, rapid, and effective tumour ablation in dogs diagnosed with STS.

Funding Information: Focused Ultrasound Foundation.

PROGNOSTIC SIGNIFICANCE OF CD25 EXPRESSION EVALUATED BY FLOW CYTOMETRY IN DOGS WITH B-CELL LYMPHOMA TREATED WITH CHOP-BASED CHEMOTHERAPY

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Introduction: Overexpression of CD25, the alpha chain of the IL2 receptor, has been associated with poor prognosis in humans with diffuse large B cell lymphoma (DLBCL). Limited data in dogs with DLBCL has been reported, with similar findings. The objective of this study was to evaluate CD25 expression as a prognostic indicator in dogs with BCL diagnosed with non-invasive diagnostics (cytology and flow cytometry) and treated with CHOP-based chemotherapy.

Methods: This was a retrospective study of 57 client-owned dogs with BCL treated with CHOP. Lymph node aspirates from dogs with a cytologic diagnosis of lymphoma comprised of intermediate to large lymphocytes were analysed with flow cytometry. Percentage of neoplastic B cells expressing CD25 and median fluorescence intensity (MFI) of CD25 were measured. Relationships of CD25 positivity and MFI with PFI and MST were evaluated along with other prognostic factors.

Results: Overall MST was 272 days (95% CI, 195–348) and median PFI was 196 days (95% CI, 171–220). High percentage of CD25 positivity ($n = 31$ dogs) was associated with longer survival time (318 vs. 176 days), which was significant in multivariable analysis ($p = .012$).

Dogs with high CD25 MFI ($n = 21$) had a longer PFI (218 vs. 140 days) although this was not significant ($p = .056$).

Conclusion: High-CD25 expression was associated with longer survival time in dogs with BCL treated with CHOP. Additional prospective studies are needed to better understand the relationship of CD25 expression with outcome in dogs diagnosed with BCL using cytology and flow cytometry.

Funding Information: N/A.

PROGNOSTIC SIGNIFICANCE OF COMPLETE RESPONSE DURING RADIATION IN FELINE SINO-NASAL LYMPHOMA A RETROSPECTIVE MULTI-INSTITUTION COHORT STUDY

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Introduction: Radiation therapy (RT) is the treatment of choice for feline sino-nasal lymphoma (fsnLSA) and the rate of local response during RT is not defined. Approximately 20%–30% will develop systemic progression within 1 year. Based on our original single institution retrospective study results, our hypothesis was that cats in complete remission (CR) at the end of RT will have shorter progression-free survival (PFS).

Methods: Multi-institutional retrospective cohort study. Inclusion criteria were histologically/cytologically confirmed fsnLSA, negative stage, treated with RT only (10 x 4.2 Gy) and at least 1-year follow-up. Tumour volumes were measured on the first and last RT cone-beam CT/MVCT. CR was defined as >90% reduction in volume. PFS was defined from first RT to systemic/local progression or death. Kaplan/Meier analysis and Cox proportional-hazards regression was performed. Cats were divided based on end of RT response: CR versus non-CR.

Results: 45 cats met the inclusion criteria. By the end of RT, 13 (29%) were in CR, 30 (67%) were in non-CR, and 2 (4%) were progressive. Overall median PFS was 470 days. The median PFS of CR was significantly shorter (213 days) compared to non-CR (681 days), hazard ratio: 3.35 (95% CI: 2.1–14.2), ($p = .0007$). At 18-months post RT, 12/13 (92%) of CR had progressed versus 12/30 (40%) of non-CR. Twelve (27%) developed systemic LSA, six (15%) had local recurrence and five (11%) had both.

Conclusion: The outcome of this cohort of cats confirms our hypothesis and suggests that CR by the end of RT is a negative prognostic factor.

RABACFOSADINE (TANOVEA) FOR THE TREATMENT OF RELAPSED MULTICENTRIC CANINE LYMPHOMA

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Introduction: The aim of this study was to evaluate the efficacy and adverse event profile of rabacfosadine for the treatment of relapsed multicentric canine lymphoma. Previous studies evaluating rabacfosadine for relapsed lymphoma have assessed selected patient populations, various doses, or with additional treatments. Therefore, data evaluating a larger number of dogs receiving standard doses of single-agent rabacfosadine in the relapse setting is needed.

Methods: This was a single arm, open-label, multi-institutional prospective trial. Dogs received rabacfosadine at 1.0 mg/kg every 21 days for up to five treatments. Response was assessed via VCOG-CTCAE criteria, and the progression free interval (PFI) was calculated by the Kaplan–Meier method. The effect of variables on PFI and overall response rate (ORR) was evaluated. Adverse events (AEs) were summarized.

Results: 159 dogs were evaluated. Most dogs were heavily pre-treated (70% received >2 previous protocols). The ORR was 46% (20% CR, 26% PR). Immunophenotype, substage and degree of pre-treatment were significantly associated with PFI. Responders (CR or PR) had a significantly longer median PFI than non-responders (118 days and 63 days for CR and PR, respectively, vs. 21 days for NR). Immunophenotype and degree of pre-treatment were significantly associated with response (ORR = 67% for dogs with B cell lymphoma at first relapse). AEs were similar to those reported in other studies.

Conclusion: These data confirm the efficacy of rabacfosadine in the relapse setting,and affirm improved efficacy in less heavily pre-treated patients with B cell disease.

Funding Information: This study was sponsored by VetDC, Elanco and the FDA (grant # 1R01 FD006323-01).

RETROSPECTIVE EVALUATION OF TOCERANIB PHOSPHATE (PALLADIA®) IN TREATMENT OF FELINE PANCREATIC CARCINOMA

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Introduction: Feline exocrine pancreatic carcinoma is a rare cancer with generally poor prognosis in cats. Historical management has included surgery and/or injectable chemotherapy. The primary aim of this study was to evaluate the biological response in cats with pancreatic carcinoma treated with toceranib phosphate (TP). As a secondary aim, the progression free interval (PFI) and median survival time (MST) were calculated. Adverse events following treatment with TP were also evaluated.

Methods: A multi-institutional retrospective study was performed. For inclusion, cats were required to have a confirmed diagnosis of exocrine pancreatic carcinoma either by histopathology, cytology, or both, and to have received treatment with TP.

Results: Twenty-nine cats from 15 institutions were included. Twenty cats were treated for gross disease, six for microscopic/incomplete margins, two in a rescue setting, and one for maintenance after completing chemotherapy. Eleven (38%) cats had documented metastatic disease at time of diagnosis. Six cats received chemotherapy (carboplatin [5], cyclophosphamide [1]) prior to TP. One cat received chlorambucil concurrent with TP. Response data were available in 26 cats. Clinical benefit (CR, PR, SD > 10 weeks) was observed in 13/26 (50%; CR = 3; SD = 10). Adverse events (AEs) were observed in 9/29 (31%) of cats. Most AEs were low grade (VCOG) and successfully managed with supportive care and/or dose adjustments.

Conclusion: Although feline exocrine pancreatic carcinoma continues to be a difficult disease to treat, TP appears to provide clinical benefit. Prospective studies are warranted to further evaluate the potential clinical utility of TP in management of this cancer.

SENSITIVITY OF CANINE HAEMATOLOGICAL MALIGNANCIES TO BH3 MIMETICS

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Introduction: The small molecule BH3 mimetic venetoclax (VEN) leads to rapid apoptosis of human haematological cancers via BCL2 inhibition and is approved for treatment of relapsed/refractory chronic lymphocytic leukaemia (CLL) and acute myeloid leukaemia. In dogs, BCL2 is diffusely expressed in B-cell lymphoma, and may be over-expressed in peripheral T-cell lymphoma. This study assessed the ex vivo viability of primary canine haematological cancer cells following treatment with VEN and the dual BCL2/BCLxL inhibitor, navitoclax (NAV).

Methods: Peripheral blood mononuclear cells and/or lymph node and/or bone marrow samples were collected from dogs with lymphoma (LSA), leukaemia and multiple myeloma (MM) from July 2019–March 2021. Cells were incubated with BH3 mimetics for 24 hours at various concentrations (2–10 000 nM). Viable cells were enumerated by flow cytometry using propidium iodide exclusion and surface staining of CD3 and CD21. Half maximal effective concentration (EC50) was determined.

Results: Nineteen dogs were included in the final analysis: six intermediate-large B cell LSA, three peripheral T-cell lymphoma (PTCL), four acute leukaemias, two T-cell CLL, two null-cell large cell LSA, one T-zone LSA (TZL) and one MM. All dogs with PTCL, TZL and T-CLL showed marked sensitivity to VEN and NAV, whilst all intermediate to large B cell LSA, MM and null cell LSA cells were resistant to VEN. Sensitivity in acute leukaemia varied, though three samples were sensitive to NAV.

Conclusion: Canine T cell malignancies are killed by BH3 mimetics at concentrations achievable in vivo, thus VEN may be a novel therapeutic agent for treatment of these diseases.

Funding Information: Funding for the project was provided, in part, by The Canine Research Foundation and Dogs Victoria, and the University of Melbourne Resident Research Grant.

SERUM THYMIDINE KINASE 1 ACTIVITY AS A PROGNOSTIC BIOMARKER IN DOGS WITH CHOP-TREATED DIFFUSE LARGE B-CELL LYMPHOMA

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Introduction: Diffuse large B-cell lymphoma (DLBCL) is frequently treated with CHOP-based chemotherapy, which induces remission in 80%–95% of cases. However, not all dogs derive meaningful benefit

from CHOP and prognostic factors for dogs with DLBCL are poorly defined. Serum thymidine kinase 1 (TK1) activity, a marker of tumour cell proliferation, has shown promising initial results as a prognostic biomarker in dogs with multicentric lymphomas. The purpose of this study was to determine if baseline serum TK1 activity is associated with clinical outcome in dogs with CHOP-treated DLBCL.

Methods: Baseline serum TK1 activity was measured in banked sera from 100 dogs with CHOP-treated DLBCL using a commercially available ELISA kit. Data on other potential prognostic factors were obtained retrospectively from electronic medical records. Multivariable statistical methods were used to find associations between potential prognostic factors and progression-free survival (PFS) and attainment of complete remission.

Results: TK1 activity at baseline was not associated with reduced PFS ($p = .299$) or attainment of complete remission ($p = .910$) following CHOP chemotherapy. Of the other prognostic factors analysed, only purebred (vs. mixed breed) status (HR 8.81, 95% CI 1.68–46.30, $p = .010$), attainment of complete (vs. partial) remission (HR 0.09, 95% CI 0.02–0.49, $p = .006$), and baseline serum C-reactive protein concentration (HR 1.19, 95% CI 1.07–1.32, $p = .001$) were independently associated with PFS.

Conclusion: Based on these findings, baseline serum TK1 activity does not appear to be a useful prognostic biomarker in dogs with CHOP-treated DLBCL.

Funding Information: Veterinary Cancer Society–Gordon Theilen Resident Research Grant.

THE PHARMACOKINETICS OF ORAL FRACTIONATED CYCLOPHOSPHAMIDE IN SEVEN CANCER-BEARING DOGS

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Introduction: Cyclophosphamide (CP) is an alkylating chemotherapeutic included in many treatment protocols for canine cancer. CP requires hepatic metabolism for activation to the intermediate compound 4-hydroxycyclophosphamide (4-OHCP) which then spontaneously forms alkylating phosphoramide mustard. The total dose of CP is frequently fractionated and given over multiple days. CP is reported to cause auto-induction of metabolism in humans, with faster CP clearance and relatively increased 4-OHCP formation following fractionated versus bolus dosing, however canine pharmacokinetic studies of CP dose fractionation are lacking.

Methods: The study objective was to evaluate the pharmacokinetics of fractionated oral CP at the standard dose of 200–250 mg/m^2 over multiple days in a prospectively identified population of cancer-bearing dogs. Plasma concentrations of CP and 4-OHCP were measured by ultra-high performance liquid chromatography tandem-mass spectrometry in seven dogs following the first and last pills to assess for auto-induction of CP metabolism.

Results: A non-parametric, paired T-test of elimination rate values showed no significant difference in the rate of CP elimination between first and last doses (0.73 ± 0.46 hr-1 vs. 1.22 ± 0.5 hr-1; $p = .125$). Additionally, no significant difference in dose-normalized 4-OHCP exposure was identified between first and last doses (5.9 ± 2.1 hr*ng/ml vs. 7.9 ± 6.4 hr*ng/ml; $p = .936$).

Conclusion: These results suggest that fractionated dosing may not increase exposure to the active metabolite of CP in dogs as it does in humans. As such, oral bolus and fractionated dosing may be equivalent in terms of bio-activation of CP in dogs administered standard dosing of 200–250 mg/m^2.

Funding Information: Supported by the Center for Companion Animal Health, School of Veterinary Medicine, University of California, Davis.

APPLICATION OF LIQUID BIOPSY TECHNOLOGY TO DETECT LYMPHOMA IN DOGS

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Introduction: Lymphoma is one of the most common canine cancers with an estimated incidence of 20–100 cases per 100 000 dogs. Liquid biopsy technology offers a novel, non-invasive approach to the identification and classification of lymphoma in dogs.

Methods: Blood samples from 57 dogs with a confirmed diagnosis of lymphoma were subjected to DNA extraction, proprietary library preparation and next-generation sequencing. Sequencing data were analysed using an internally developed bioinformatics pipeline to detect genomic alterations associated with the presence of cancer. The testing laboratory was blinded to the cancer status and type of cancer present in these patients until after test results were issued.

Results: In 57 dogs with lymphoma, the liquid biopsy test returned a positive result for 47 cases, yielding an overall sensitivity of 82%. When analysed by subtype, the test showed 78% sensitivity for B-cell lymphoma cases (25/32), 100% for T-cell lymphoma (9/9), 50% for both B- and T-cell (1/2), and 86% for type 'unknown' (12/14). Furthermore, in the 47 screen positive cases, a 'cancer signal origin' prediction of "lymphoma" was provided in 19 cases. Among tested dogs with a confirmed cancer diagnosis other than lymphoma, none had a 'cancer signal origin' prediction of lymphoma.

Conclusion: A novel, blood-based canine cancer screening test was successful at detecting genomic alterations associated with cancer in 82% of dogs with a diagnosis of lymphoma. Additionally, the screening test was able to identify patterns of genomic abnormalities that

were predictive of lymphoma in 40% of lymphoma-diagnosed cases that received a 'cancer signal detected' test result.

Funding Information: This study received funding from PetDx.

CLINICAL VALIDATION OF A MULTI-CANCER EARLY DETECTION (MCED) BLOOD-BASED LIQUID BIOPSY TEST IN DOGS USING NEXT-GENERATION SEQUENCING (NGS)

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Introduction: Cancer is the leading cause of death in dogs, with no established screening paradigms for early detection. As a result, many patients are diagnosed at an advanced stage, when clinical signs have developed and prognosis is poor. Liquid biopsy methods that interrogate cancer-derived genomic alterations in cell-free DNA fragments in blood are being adopted for early cancer detection in human medicine. Development and use of a non-invasive test for the early detection of cancer in dogs may lead to improved outcomes.

Methods: Blood samples from an all-comers cohort of 191 cancer-diagnosed dogs and 188 presumably cancer-free dogs were subjected to DNA extraction, proprietary library preparation and next-generation sequencing. Sequencing data were analysed using an internally developed bioinformatics pipeline to detect genomic alterations associated with the presence of cancer.

Results: The overall test sensitivity in cancer-diagnosed subjects was 48% (92/191). Of 188 samples from presumably cancer-free dogs, 180 tested negative ('putative true negatives') and eight tested positive ('putative false positives,' pFP). In at least 2 pFP cases, patients were diagnosed with cancer 6 and 7 months following blood collection and were excluded from final performance analyses, resulting in a minimum test specificity of 97%.

Conclusion: A novel, multi-cancer early detection (MCED) liquid biopsy test has demonstrated the ability to identify cancer-associated genomic markers (in some cases months prior to the onset of clinical signs) in canine patients, with sensitivity and specificity comparable to commercially available MCED testing options in humans. Early detection and treatment of cancer are key determinants of optimal clinical outcomes.

Funding Information: This study received funding from PetDx.

CLINICAL VALUE OF CARCINOEMBRYONIC ANTIGEN IN MAMMARY NEOPLASMS OF BITCHES

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Introduction: This study aimed at evaluating the behaviour and understanding the diagnostic value of the carcinoembryonic antigen (CEA) in bitches with mammary carcinoma as a tool for monitoring and prognosis of canine cancer patients.

Methods: Serum samples from 77 bitches were divided into four groups, G1 ($n = 21$), control group (healthy/neoplasia free bitches); G2 ($n = 31$), bitches with non-metastatic mammary carcinoma less than 3 cm; G3 ($n = 12$), bitches with non-metastatic mammary carcinoma greater than 3 cm; and, G4 ($n = 13$) bitches with mammary carcinoma and lymph node metastasis. The marker was dosed once in G1, whereas in G2, G3 and G4, CEA levels were determined before (M0) and 15 days after (M1) mastectomy, using the ELISA kit for humans while reading used ELISYS ONE human. A group of 11 bitches was followed up 45 days after mastectomy (M2).

Results: CEA values increased significantly in bitches with mammary carcinoma, metastatic tumours with a diameter larger than 3.0 cm and high grade, compared with healthy ones. In addition, mastectomy reduced the CEA concentration in the blood ($p < .05$) whereas high-CEA levels were associated with unfavourable prognostic factors ($p < .05$). The biomarker presented good diagnostic value, especially for more aggressive tumours.

Conclusion: In conclusion, CEA serum concentrations allowed to follow efficiently the evolution of mammary tumours in bitches, since CEA values increased in bitches with mammary gland tumour and decreased after mastectomy while correlating with prognostic factors such as tumour size, nodal metastasis and histological grade.

Funding Information: Fundação de Amparo à Pesquisa do Estado de São Paulo, Grant/Award Numbers: 2015/15015-9, 2016/00128-5.

COMBINE RADIATION AND IMMUNOTHERAPY AS A TREATMENT MODALITY OF CANINE ORAL MELANOMA

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Introduction: Canine oral melanoma remains the most common malignant oral tumour; the primary treatments are wide-margin surgery and radiation therapy (RT). In human melanoma, several immunotherapies were approved, and recent studies also focus on the combination of immunotherapy and RT. Less is known about the combination treatment; thus, this research hypothesizes that combining immunotherapy and RT will provide clinical benefit.
Methods: The immunotherapy used is the dendritic cell/tumour cell fusion vaccine. Dendritic cells are separated and cultured from healthy dog's blood, then fused with tumour cells from canine patients. Hypofractionated RT was utilized, with 7.5–8.5 Gy, weekly in five fractions, and regional lymph node included. Vaccination will start after the second radiation treatment. A total of four doses was administered 2 weeks intervals. Response and adverse effects will be recorded and monitored by physical and haematological examination.
Results: Five dogs were recruited. All had CR or PR after RT, 1 had lung metastasis 4 weeks after last RT. Two had local recurrence 79 days, and 219 days after initial RT. Four patients died, and one is still alive. Three died of tumour-related reasons at 125, 152 and 293 days after diagnosis. One had sudden death without local or distant progression at 83 days after diagnosis. The alive one patient is progression-free now, follow-up 674 days.
Conclusion: Though the case number is low, the effect of combination therapy still warrants further investigation. Detailed cytokine changes and cell analysis will provide us more information.
Funding Information: The study has no funding.

DETECTION OF CANINE TRANSITIONAL CELL CARCINOMA OF THE BLADDER BY CANINE OLFACTION

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Introduction: The objective of this study was to investigate whether trained dogs can scent tumour-related volatile compounds in dogs' urine and accurately detect canine urinary transitional cell carcinoma (TCC).
Methods: Urine samples from healthy dogs without urinary tract abnormalities (control), dogs with non-malignant urinary tract disease (control), and dogs with urinary TCC (positive) were collected prospectively. All dogs underwent bladder ultrasound and urinalysis. Urine samples were frozen in glass jars until analysis. TCC was diagnosed upon cytology and/or histopathology. Dogs were naïve of chemotherapy, NSAIDs was permitted.
Dogs previously trained to detect human bladder and prostate cancer were presented with randomly allocated sample lines of 2 control samples, 1 positive sample and 1 biological sample irrelevant to this study (filler), or lines of only control and filler samples. Dog handlers were blinded to the samples identity until the dog's behaviour had been recorded, after which the data collection software revealed the correct response. The dogs were rewarded when correct.
Results: Three trained dogs were tested under double-blind conditions and challenged with 60 controls, 30 fillers and 30 TCC samples. Overall, no fillers were incorrectly indicated, 8.3% of controls were incorrectly indicated and 80% of TCC were correctly indicated. The difference in the proportions of control and target samples indicated was highly significant for all three dogs (overall p value <.001).
Conclusion: This study suggests that dogs can be trained to detect canine TCC in urine and could lead to the development of an electronic nose as an additional diagnostic test for TCC.
Funding Information: No conflict of interest or funding.

DEVELOPMENT OF A CANINE ANTI-CANINE PD-1 ANTIBODY FRAGMENT TO ENHANCE CART EFFICIENCY IN SOLID TUMOURS

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Introduction: Chimeric antigen receptor (CAR) T cells have shown remarkable efficiencies in haematological malignancies however, their effectiveness in solid tumours is disappointing. One contributing factor might be the negative influence of checkpoints on CART cells in the tumour microenvironment. Engagement of programmed cell death protein-1 (PD-1) on T cells by PD-L1 inhibits effector function. We hypothesized that a combination of PD-1:PD-L1 inhibition with CART cells could increase their effectiveness in solid tumours. Inhibitory canine scFv antibody fragments will be employed in next generation CART cell design to generate CART that constitutively secrete anti-PD1 to increase efficiency in solid tumours.
Methods: Canine (c) PD-1-specific scFvs isolated from a fully canine scFv phage display library were assessed in an ELISA-based assay for their ability to inhibit cPD-1/cPD-L1 interaction. Inhibitory clones were reformatted as full-length monoclonal antibodies and their affinity for

cPD-1 was analysed by surface plasmon resonance, and their ability to detect cPD-1 in FFPE specimens was determined by IHC.

Results: Three anti-PD1 scFv clones strongly inhibited cPD-1/cPD-L1 interactions. Full-length antibodies were generated for two clones, which showed single digit and subnanomolar binding affinities for cPD-1. One clone detected cPD-1 expression in FFPE lymph node specimens. Preliminary data suggests that this clone can also enhance canine effector T cell function following mitogen activation in vitro.

Conclusion: We have identified 2 anti-cPD-1 clones that have potential for use either as a mAb or as scFv secreted from next generation CART cells to enhance effector T cell function in solid tumours.

Funding Information: V Foundation for Cancer Research, NCI/NIH/SBIR Contract 75N91018C000042, JSPS KAKENHI Grant Number 21 J01155.

DEVELOPMENT OF AN EXOSOMAL GENE SIGNATURE TO DETECT MINIMAL RESIDUAL DISEASE IN DOGS WITH OSTEOSARCOMA USING A NOVEL XENOGRAFT PLATFORM AND MACHINE LEARNING

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Introduction: Osteosarcoma, the most common primary bone tumour in both dogs and humans, has a guarded prognosis. A major hurdle in developing more effective therapies is the lack of disease-specific biomarkers to predict risk, prognosis, or therapeutic response. Exosomes are secreted extracellular microvesicles emerging as powerful diagnostic tools. However, their clinical application is precluded by challenges in identifying disease-associated cargo from the vastly larger background of normal exosome cargo.

Methods: We developed a method using a xenograft model to distinguish tumour-derived from host-response exosomal mRNAs, allowing for identification of canine osteosarcoma-specific gene signatures by RNAseq and a species-differentiating bioinformatics pipeline.

Results: An osteosarcoma-associated signature consisting of five gene transcripts (SKA2, NEU1, PAF1, PSMG2 and NOB1) was validated in dogs with spontaneous osteosarcoma by qRT-PCR with machine learning. Serum/plasma exosomes were isolated from 53 dogs in distinct clinical groups ('healthy,', 'osteosarcoma,' 'other bone tumour,' or 'non-neoplastic disease'). Pre-treatment samples from osteosarcoma cases were used as the training set and a validation set from post-treatment samples was used for testing, classifying as 'osteosarcoma–detected' or 'osteosarcoma–NOT detected.' Dogs in a validation set whose post-treatment samples were classified as 'osteosarcoma–NOT detected' had longer remissions, up to 15 months after treatment.

Conclusion: We identified a gene signature predictive of molecular remissions with potential applications in the early detection and minimal residual disease settings. These results provide proof-of-concept for our discovery platform and its utilization in future studies to inform cancer risk, diagnosis, prognosis and therapeutic response.

Funding information: Request information from author.

EARLY DETECTION OF CANCER IN PRESUMABLY CANCER-FREE DOGS USING BLOOD-BASED GENOMIC ANALYSIS

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Introduction: Early detection is the primary goal of effective cancer screening. In dogs, traditional screening protocols have relied mostly upon the patient's medical history and routine physical examination, often resulting in identification of cancer at advanced stages following the development of clinical signs. As technology advances, novel screening paradigms are emerging. One such advancement involves the analysis of cancer-associated analytes in blood, which may aid in the detection of malignancy prior to the emergence of clinical signs, potentially allowing for earlier diagnosis and treatment.

Methods: Blood samples from presumably cancer-free dogs were subjected to DNA extraction, proprietary library preparation and next-generation sequencing (NGS). Sequencing data were analysed using an internally developed bioinformatics pipeline to detect genomic alterations associated with the presence of cancer.

Results: In 188 samples from presumably cancer-free dogs, there were 180 screen negative ('true negative') and eight screen positive ('putative false positive,' pFP) results. Follow-up information was available for at least five pFP cases; in two of these cases, patients were diagnosed with cancer 6–7 months following blood collection, one patient died at age 12 of unknown causes, and workup for two patients found no evidence of cancer. Follow-up is actively being pursued for the remaining three pFP cases.

Conclusion: Advances in genomic sequencing technologies are enabling the development of novel cancer screening tools in veterinary medicine. One approach, involving blood-based NGS, may identify early genomic markers of cancer months prior to the development of clinical signs. Early identification and treatment of cancer are important factors to optimize patient outcomes.

Funding Information: This study received funding from PetDx.

EFFECTS OF CO-ADMINISTRATION OF MOLECULAR TARGET DRUGS IN CANINE HISTIOCYTIC SARCOMA CELL LINES

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Introduction: Canine histiocytic sarcoma (CHS) is characterized by aggressive biological behaviour, and the establishment of effective chemotherapy for this disease is needed. In our previous study, the FGFR1-ERK/Akt pathway was suggested to be activated in CHS tissues, but not all CHS cell lines were sensitive to single administrations of FGFR1 inhibitors. We hypothesized that the antitumor effect would be enhanced by co-administration of drugs targeting FGFR1-ERK/Akt pathway in CHS cell lines.
Methods: Three molecular targeted drugs against FGFR1-ERK/Akt pathway, dasatinib, trametinib, and ponatinib, were selected to investigate antitumor effects in 10 CHS cell lines. Enhancements of inhibitions of cell proliferation by co-administrations of two drugs were evaluated using combination index, an index of pharmacodynamic drug interactions. Changes in protein phosphorylation involved in the ERK/Akt pathway were evaluated using Western blotting.
Results: In two of the 10 cell lines, co-administration of drugs was not examined because they were sensitive to the single administration of dasatinib (IC50: 10.6/50.6 nM). In the other eight cell lines, co-administration of dasatinib and trametinib showed the highest synergic effects with CI values ranging from 0.01 to 1.01. Decreases of both phosphorylated ERK and Akt were observed when synergic effects were observed by co-administration of these drugs.
Conclusion: In CHS, the co-administration of dasatinib and trametinib enhanced the antitumor effect compared to single administrations through inhibiting ERK/Akt activities. Further studies are needed to investigate the efficacies and adverse events of the co-administration in CHS cases.

EFFICACY AND TOLERABILITY OF SEQUENTIAL COP AND DOXORUBICIN CHEMOTHERAPY PROTOCOL FOR TREATMENT OF LYMPHOMA IN CATS

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Introduction: Chemotherapy protocols containing cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) are commonly utilized for the treatment of high-grade lymphoma in cats. The aim of this study was to evaluate tolerability and efficacy of a COP/DOX protocol, where cats were intended to receive COP weekly for 6 weeks followed by doxorubicin every 3 weeks for six doses.
Methods: Records were retrospectively reviewed for cats with lymphoma treated at the University of Missouri from 2005 to 2020. Cats who received sequential COP and doxorubicin were included. Data collected included signalment, method of diagnosis, tumour location, staging and baseline diagnostics, drug dosages, adverse events, re-staging diagnostics and treatment response. Kaplan–Meier analysis was used to estimate median survival time (MST) and progression free interval (PFI).
Results: Twenty-one cats met inclusion criteria. Lymphoma localized to the gastrointestinal tract in 47.6% of cats. The number of cats achieving clinical remission (CR) and partial remission (PR) throughout COP were 8 (38.1%) and 8 (38.1%), respectively. Of the five PR cats at the completion of COP, one achieved CR on doxorubicin, for an overall response rate of 85.7%. Three cats did not respond to either drug. PFI was 87 days (range 29–998) and MST was 151 days (range 49–2207). High-grade adverse events included grade 4 neutropenia (COP: $n = 3$), grade 3 vomiting (doxorubicin: $n = 1$), and grade 3 anorexia (COP: $n = 1$, doxorubicin: $n = 1$).
Conclusion: Toxicity, response rates, PFI and MST are similar to previous reports. This COP/DOX protocol is an acceptable treatment option for cats with lymphoma.

EGFR PEPTIDE VACCINATION INDUCES EGFR/HER2-SPECIFIC IMMUNITY IN DOGS WITH SOLID TUMOURS

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Introduction: EGFR and HER2 are overexpressed by human and canine malignancies and are negative prognostic indicators. Vaccinating mice with a cryptic self-peptide from the EGFR extracellular domain, also present in HER2 and HER3, induced potent humoral and cell-mediated responses specific for EGFR and HER2 on growing tumours. (1) The primary objective of this study was to determine if vaccination with the homologous canine cryptic EGFR peptide triggered comparable responses in dogs with EGFR-expressing solid

tumours. Secondary objectives included monitoring adverse events and 1-year survival times.

Methods: Dogs with solid tumours (including 43 osteosarcomas) presented to a veterinary oncology practise were enrolled after owners signed informed consent, approved by the Yale University IACUC. Tumour histopathologies were confirmed by veterinary pathologists. Following standard-of-care treatment, for example, limb amputation/carboplatin, dogs received two subcutaneous vaccinations, 3-weeks apart, with canineEGFR p527 peptide ≥3 weeks post-chemotherapy, and serial serum samples collected: Day 0-serum+vaccine #1; Day 21-serum+vaccine #2 (boost); Day 40–50 serum (convalescent); Day >50 serum (long-term). Standard immunologic assays to detect antibodies (ELISA), EGFR/HER2 expression, antibody and immune cell infiltrates (flow cytometry, immunofluorescence), intracellular signalling (Western blot), and cell proliferation (3H-thymidine incorporation) were employed. Statistical differences between in vitro conditions were analysed using the Mann–Whitney test.

Results: Immunization with canineEGFR p527 generated canine antibodies that bound canine and human EGFR, and HER2. Antibodies inhibited EGF signalling, promoted antibody and CD8 T-cell infiltration into the tumour microenvironment, and inhibited tumour cell growth in vitro.

Conclusion: EGFR peptide vaccination overcomes immune tolerance by triggering robust humoral and cell-mediated, specific immune responses in EGFR+-tumour-bearing dogs.

Funding Information: (1) Hester A. Doyle, Raymond A. Koski, Nathalie Bonafé, Ross A. Bruck, Stephanie M. Tagliatela, Renelle J. Gee, Mark J. Mamula: Epidermal growth factor receptor peptide vaccination induces cross-reactive immunity to human EGFR, HER2 and HER3. Cancer Immunol Immunother 67 (10):1559–1569, 2018. These studies were supported by awards from the Canine Cancer Research Alliance (MJM) and The MJ and Caral G Lebworth Foundation (MJM). No conflicts of interest.

EVALUATION OF BRAIN TUMOUR RESECTION WITH HYPOFRACTIONATED RADIOTHERAPY: RETROSPECTIVE STUDY

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Introduction: Tumour resection is effective for intracranial tumours. However, recurrence may occur early after resection. Chemotherapy and radiotherapy are administered for residual tumour cells. Radiotherapy, including hypofractionated radiotherapy, may be effective for intracranial tumours. The purpose of this study was to evaluate brain tumour resection with hypofractionated radiotherapy.

Methods: Dogs with intracranial tumours underwent resection by the same surgeon; some dogs received radiotherapy and/or chemotherapy. Dogs with hematomas and meningiomas were excluded. Kaplan–Meier survival curves and log-rank analysis were used to compare the presence and absence of radiotherapy.

Results: Surgery was performed in 18 dogs; postoperative radiotherapy and chemotherapy were administered in seven and four dogs, respectively. The tumour locations were the cortex ($n = 17$) and brainstem ($n = 1$). The diagnoses were anaplastic oligodendrocyte ($n = 6$), glioblastoma ($n = 3$), granulomatous meningoencephalitis ($n = 3$), histiocytic sarcoma ($n = 2$), glioma ($n = 2$), oligodendrocyte tumour ($n = 1$), and choroid plexus papilloma ($n = 1$). Chemotherapy drugs were not administered in dogs that received radiotherapy. A median of 26 Gy of radiotherapy in four fractions was administered for 1 month. The overall survival times in dogs that underwent surgery with/without chemotherapy, with chemotherapy, and with radiotherapy were 2.5, 5.5, and 13 months, respectively. Acute and late radiation-associated adverse events were not noted. Dogs that received radiotherapy survived significantly longer than dogs that underwent surgery with/without chemotherapy ($p < .01$).

Conclusion: Surgery and postoperative hypofractionated radiotherapy might be effective for central nervous system tumours.

Funding Information: No.

EVALUATION OF A BLOOD-BASED LIQUID BIOPSY TEST IN CANINE PATIENTS WITH DIFFICULT-TO-DIAGNOSE CANCERS

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Introduction: Cancer in dogs may occur in anatomical locations that are difficult or impossible to access by fine needle aspiration or surgical biopsy, making confirmation of malignancy difficult, or in some cases, impossible. These 'difficult to diagnose' (DTD) cases may include: endocrine, bone, central nervous system (CNS), intra-thoracic, intra-abdominal, upper respiratory tract, and thymic tumours. In such cases, a non-invasive 'aid in diagnosis' test may prove valuable in establishing whether the mass is malignant.

Methods: Blood samples from 191 dogs with a variety of cancer diagnoses were subjected to DNA extraction, proprietary library preparation and next-generation sequencing. Sequencing data were analysed using an internally developed bioinformatics pipeline to detect genomic alterations associated with the presence of cancer. The testing laboratory was blinded to the cancer status and type of cancer present in these patients until after test results were issued.

Results: In an all-comers cohort of 191 cancer-diagnosed subjects, 33% ($n = 63$) were designated as DTD cases, comprising over

20 distinct cancer types from the above-mentioned classes. In these DTD cases, the non-invasive test yielded a Cancer Signal Detected result in 25 cases, for an overall detection rate of 40%.

Conclusion: A non-invasive, blood-based cancer detection test has potential to serve as an aid in diagnosis for patients with DTD masses. This type of testing may provide clinical utility in situations where tissue-based diagnosis may not be available or achievable for masses that are suspected to be malignant.

Funding Information: This study received funding from PetDx.

EVALUATION OF COMBINED IN VITRO PROTEASOME INHIBITION PLUS CHOP CHEMOTHERAPY COMPONENTS IN CANINE B CELL LYMPHOMA CELLS

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Introduction: Canine multicentric lymphoma is commonly treated with CHOP (cyclophosphamide, hydroxydaunorubicin, vincristine [oncovin], prednisone) combination cytotoxic chemotherapy. Proteasome inhibitors are employed clinically in combination with CHOP for the treatment of human haematological malignancies, including lymphoma. The aim of this study was to determine the effect of proteasome inhibitors bortezomib and ixazomib on a canine B cell lymphoma cell line (CLBL-1) cell viability, and to investigate whether they sensitize cells to CHOP chemotherapy agents. We hypothesized that proteasome inhibitors will reduce CLBL-1 viability and will be synergistic with CHOP compounds.

Methods: The effects of proteasome inhibitors and CHOP on CLBL-1 viability were determined using a resazurin assay. Drugs were combined over a range of doses that maintained a IC50:IC50 ratio as well as a clinically relevant Cmax:Cmax ratio.

Results: After 48 h exposure, the IC50 of bortezomib was 15.1 nM and of ixazomib was 59.14 nM. The IC50 after 48 h exposure to the CHOP compounds cyclophosphamide (as 4-HC), hydroxydaunorubicin, and vincristine were 1.623 uM, 111.5 nM and 156.1 nM, respectively. Proteasome inhibitors plus hydroxydaunorubicin had a synergistic effect on CLBL-1 viability after IC50:IC50 co-treatment. Vincristine had an antagonistic effect at the same respective dose ratio, but when combined using a Cmax:Cmax ratio, proteosome inhibitors synergized with vincristine. We are currently investigating CLBL-1 responses to cyclophosphamide and prednisone in combination with proteasome inhibition.

Conclusion: These results may have clinical utility, as proteasome inhibition could potentially be used with a synergizing CHOP compound to improve CHOP responsiveness for canine lymphoma patients.

Funding Information: OVC Pet Trust.

EVALUATION OF INFRARED THERMOGRAPHY AS A COMPLEMENTARY DIAGNOSTIC METHOD FOR THE ASSESSMENT OF CANINE SKIN TUMOURS

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Introduction: Thermography is an innovative diagnostic tool, capable of detecting superficial temperature changes of the skin, induced by increased local inflammation and metabolism, neoangiogenesis and tissue necrosis, which frequently occur in tumours. In this study, we used infrared thermography to characterize different biological groups of skin neoplasm.

Methods: Twenty-two dogs with 34 confirmed skin neoplasms by histopatologic evaluation were enrolled, divided in nine groups: mast cell tumour (MCT–10), soft tissue sarcoma (STS–4), cutaneous carcinoma (CC–3), melanoma (M–2), perianal neoplasms (PN–4), mammary gland tumours (MGT–4), malignant trichoepithelioma (MT–1), hemangiosarcoma (HSA–2) and cutaneous benign neoplasms (CBN–4). Thermography assessed temperatures at central spot (SpT) and area (AT) of tumours and healthy skin (SpNT; ANT).

Results: Higher values of SpT (37.9°C) and AT (38.0°C) were observed in MGT and lower in HSA (30.6°C; 32.0°C). Most groups have mixed patterns of temperature along the nodule extension, even though STS had a greater number of hot tumours and PN, HSA and CBN of cold tumours. In CBN, temperature distribution was always homogeneous. MCT, STS, CC, M, PN and MT showed an increased halo of peripheral temperature, with extensions ranging from 0.6 to 6.8 centimetres.

Conclusion: Thermographic assessment of cutaneous neoplasms was a powerful tool for the complementary diagnosis of skin neoplasms, characterizing certain groups of tumours. A higher number of tumours must be analysed, but so far the method provides important information about factors of malignancy and pre-operative planning for the veterinary oncologist.

Funding Information: There were no fundings for the development of this research project.

EVALUATION OF KI67 EXPRESSION AS A PROGNOSTIC MARKER FOR CANINE NODAL SMALL B-CELL LYMPHOMA DIAGNOSED BY FLOW CYTOMETRY

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Introduction: Nodal small cell B-cell lymphoma subtypes in dogs cannot be distinguished by flow cytometry and information regarding treatment, prognosis and outcome is limited. The aim of this study was to describe clinical outcome in canine nodal small B-cell lymphoma cases as diagnosed by flow cytometry and correlate clinical, laboratory and Ki67 expression data with outcome. We hypothesized that increased Ki67 expression measured by flow cytometry may be associated with shorter progression-free interval (PFI) and overall survival times (OST).

Methods: Nodal small B-cell lymphoma cases were identified by flow cytometry by an expansion (>80%) of CD21+ B cells that were small-intermediate in size by forward light scatter. The percentage of Ki67-expressing B cells was measured by flow cytometry in all cases. Treatment and outcome data were extracted from medical records.

Results: Forty-nine cases were included. Median PFI and OST for all cases were 119 and 222 days, respectively. For dogs treated with a CHOP-based protocol ($n = 32$), median PFI and OST were 70 and 267 days, respectively. The median B-cell Ki67% was 41% (range 3%–97%). Among CHOP-treated cases, those with very low proliferation (<11 > 11% Ki67 [MST 242 days; $p = .014$]). However, this association was not significant when all cases were combined ($p = .077$; <11 > 11% Ki67 MST 176 days).

Conclusion: The majority of nodal small B-cell lymphoma cases had an aggressive course. Low Ki67 expression may be useful in identifying cases with better prognosis.

EVALUATION OF PLASMA NUCLEOSOME CONCENTRATIONS AS A TOOL FOR TREATMENT AND DISEASE MONITORING IN CANCER BEARING DOGS

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Introduction: Elevated plasma nucleosome concentrations (PNC) have been documented in newly diagnosed canine lymphoma and hemangiosarcoma patients. Nucleosomes have a short half-life in plasma, making them a useful surrogate for treatment response and remission monitoring. The objective of this study was to prospectively evaluate PNCs in dogs with a variety of cancers undergoing definitive therapy to determine the utility of this assay as a treatment and remission monitoring tool.

Methods: Dogs with a variety of newly diagnosed malignancies undergoing definitive therapy were enrolled in this study. Plasma was collected at diagnosis and at each treatment and recheck visit thereafter. Samples were processed as previously described. Data from medical records including response to treatment, laboratory data, imaging results and quality of life information was collected and compared to PNCs.

Results: Here we present a series of 20 dogs with a variety of cancers, including haematopoietic, mesenchymal and epithelial tumours, with PNCs that mirror treatment response and disease progression. The median PNC at diagnosis for all dogs was 203.7 ng/ml. PNCs at clinical remission (CR) or best clinical response was 48.6 ng/ml. All dogs achieving a CR had PNCs that dropped into the healthy range. Elevated nucleosome concentrations were identified after treatment delays in cases of lymphoma and prior to clinical progression in some lymphoid and non-lymphoid malignancies.

Conclusion: Plasma nucleosome concentrations can mirror treatment response and disease progression for a variety of malignancies in canines and may serve as a useful monitoring and potentially actionable tool for cancer patients undergoing treatment.

Funding Information: Funding for this study was provided in part by Volition America and by the Fred and Vola Palmer Chair for Comparative Oncology.

EXAMINING BONA FIDE IMMUNOGENIC CELL DEATH INDUCING CYTOTOXIC AGENTS AND IMMUNE PHENOTYPES IN CANINE ORAL MELANOMA

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Introduction: Immunotherapy has been heralded as the fourth pillar of cancer treatment and promises to improve outcomes for immunogenic tumours like canine oral malignant melanoma (cOMM). Multiple factors contribute to impactful immunotherapeutic responses including the immune profile of a tumour with 'immune inflamed' phenotypes associated with superior outcomes. Immunogenic cell death (ICD) is a form of specialized programmed death that promotes immune 'awakening' and generation of "immune inflamed" profiles through damage associated molecular patterns (DAMPS; calreticulin, ATP, HMBG1) induced by bona fide ICD chemotherapeutics. The evaluation of ICD-inducing agents and characterization of immune profiles in cOMM would generate provocative findings and promote the rational design of chemoimmunotherapeutic strategies for improving outcomes in pet dogs.

Methods: Three putative ICD-inducing agents (Doxorubicin, Oxaliplatin, and Bortezomib) were evaluated in three immortalized cOMM cell lines for inducing canonical markers of ICD, being calreticulin membrane translocation, ATP and HMGB1 release via confocal microscopy, luminescent ATP assay, and western blot,

respectively. The immune profile of 50 cOMM samples were characterized using immunohistochemistry for Iba1, PAX5, CD3 and FOXP3.
Results: Doxorubicin and Oxaliplatin promoted ICD in cOMM cell lines as demonstrated by calreticulin translocation (ER to plasma membrane), as well as HMGB1 release. Immune profiling of cOMM samples identified robust macrophage infiltration within all samples, with lesser and heterogeneous quantities of TILs and minimal Tregs.
Conclusion: Doxorubicin and Oxaliplatin are bona fide ICD inducing agents in cOMM and should be included into future designed chemoimmunotherapeutic protocols. Overall, cOMM samples possess inflamed profiles with predominance of macrophage infiltration.
Funding Information: Morris Animal Foundation.

GOLD NANOPARTICLES AND PHOTOTHERMAL ABLATION THERAPY AS A POSSIBLE NOVEL APPROACH FOR TREATING CANINE SOFT TISSUE SARCOMAS

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Introduction: Nanoparticles have a variety of uses in oncology. Specialized gold nanoparticles have been designed to passively accumulate in tumour tissue and maximally absorb near-infrared laser light converting it to heat resulting in selective hyperthermic cell death while sparing adjacent healthy tissues.
This pilot study was examining the effect of utilizing gold nanoparticles and laser induced photothermal heating (nanotherapy) in a spontaneous tumour model for canine soft tissue sarcomas (STS).
Methods: Ten dogs with STS varying in size, grade and location, were entered into a study over 18 months. Some of these tumours were advanced or recurrent after other therapies and were not excluded. Patients received a systemic intravenous infusion of nanoparticles followed by the application of 808 nm laser light using a diode laser with a specialized sapphire treatment probe 24 h later. Some patients received multiple treatment applications of laser light over a period of several weeks. Patient responses and any toxicities encountered were documented.
Results: At the time of this abstract, all dogs achieved either a visible partial response (30%) or complete response (70%) to therapy and experienced only minimal toxicities. The median progression free interval (PFI) was not reached in all treated dogs; however, if all patients developed recurrence at the time of this abstract, the predicted median PFI would be 289 days.
Conclusion: Nanotherapy can be used in canine STS patients with a low risk of toxicities and while providing anticancer effects. Further studies are ongoing in this patient population as well as other tumours.
Funding Information: This research was funded by Companion Animal Health in clinical patients.

HIGH-FREQUENCY IRREVERSIBLE ELECTROPORATION-INDUCED BRAIN TUMOUR CELL DEATH HAS DISTINCT EFFECTS ON BLOOD–BRAIN BARRIER ENDOTHELIUM

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Introduction: Brain tumour treatments are hindered by the blood–brain barrier (BBB), which shelters neoplastic cells. High-frequency irreversible electroporation (H-FIRE) is a minimally invasive, nonthermal ablative therapeutic in clinical trials for canine primary brain tumours. H-FIRE precisely ablates brain tumours while transiently disrupting peritumoral BBB, enhancing therapeutic delivery to infiltrative margins. H-FIRE-induced cell death and BBB disruption mechanisms remain incompletely characterized. We hypothesize that H-FIRE-induced tumour cell death induces BBB disruption.
Methods: F98 glioma, LL/2 Lewis lung carcinoma and bEnd.3 cerebral endothelial cell lines modelled primary and metastatic brain cancer and BBB endothelium, respectively. Cell membrane permeability and chromatin condensation of cancer cells were temporally measured via flow cytometry. Endothelial cells were exposed to H-FIRE-treated cancer cell supernatants, and endothelial response was assessed via flow cytometry and RT-qPCR of tight junction genes.
Results: Cancer cells exhibited dose-escalating permeabilization and chromatin condensation, with recovery occurring at lower voltages. Endothelial cell exposure to H-FIRE-ablated cancer cell supernatants resulted in rapid detachment and morphologic changes with no endothelial tight junction gene expression changes induced by immediate-post-treatment supernatants. 24 h-post-treatment F98 supernatants induced downregulation of claudin-3 and upregulation of claudin-5, while 24 h-post-treatment LL/2 supernatants induced downregulation of MPP6, SPTB, and VAPA genes.
Conclusion: Chromatin condensation implicates apoptosis in H-FIRE-induced cell death. Cerebral endothelial cell changes induced by H-FIRE tumour cell ablation suggest an indirect BBB disruption mechanism involving tight junction-associated genes.
Funding Information: Grayton Friedlander Memorial Fund.

HIGH-THROUGHPUT SEQUENCING AND CHARACTERIZATION OF THE IMMUNOGLOBULIN REPERTOIRE IN HEALTHY DOGS AND DOGS WITH B-CELL LYMPHOMA

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Introduction: Profiling the adaptive immune repertoire using high-throughput sequencing (HTS) methods has become common in human medicine, showing promise in characterizing clonal expansion of B cell receptors (BCRs) in patients with lymphoid malignancies. In contrast, most work evaluating BCR repertoires in dogs has employed traditional PCR-based approaches analysing the IgH locus only. The objectives of this study were to: (1) describe a novel HTS protocol to evaluate canine BCRs; (2) develop a bioinformatics pipeline for processing canine BCR sequencing data and (3) apply these methods to derive insights into BCR repertoires of healthy dogs and dogs undergoing treatment for B-cell lymphoma.
Methods: We isolated RNA from PBMCs of healthy dogs ($n = 25$) and dogs newly diagnosed with intermediate-to-large B-cell lymphoma ($n = 18$) with intent to pursue chemotherapy. 5′ rapid amplification of cDNA ends (5′RACE) and next-generation sequencing were employed to generate cDNA libraries and evaluate BCR repertoires. We developed an analysis pipeline to process, identify and quantify these repertoires.
Results: In healthy controls, we observed BCR sequence properties similar to what has been previously observed, supporting the accuracy of our methods. Additionally, we observed a bimodal pattern in IGHV usage, where repertoires were dominant in either IGHV3-38 or IGHV4-1. Among dogs with lymphoma, we demonstrated an ability to quantify clonal expansion pre-treatment and contraction post-treatment.
Conclusion: The tools we developed represent novel resources to better understand canine haematopoietic malignancies. Future studies employing these tools may improve disease tracking, provide earlier recognition of relapses and ultimately offer better-informed and targeted therapeutics.
Funding Information: University of Minnesota Academic Health Center Faculty Seed Grant Program. Biotechnology and Biological Sciences Research Council Doctoral Training Partnership. Wellcome Trust.

IMMUNOCIDIN® NON-SPECIFIC IMMUNOTHERAPY WITH CONCURRENT DOXORUBICIN FOR CANINE SPLENIC HEMANGIOSARCOMA

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Introduction: Hemangiosarcoma (HSA) of the canine spleen is an aggressive, chemotherapy resistant tumour, and despite surgery and chemotherapy, most patients succumb to their disease within 6 months. New treatment modalities, such as immunotherapy, may offer a therapeutic advantage. Immunocidin is a USDA-licenced, mycobacterial cell wall fraction, non-specific immunotherapeutic with preliminary intravenous (IV) safety data in dogs with various cancers. The study aims were to evaluate safety of combination doxorubicin and Immunocidin, and the impact of Immunocidin on overall survival time (OST).
Methods: Patients with histologically diagnosed splenic HSA were prospectively enrolled to receive five doses of IV doxorubicin (30 mg/m^2) and IV Immunocidin every 2 weeks. Adverse events (AEs) were graded according to the VCOG CTCAE scheme. OST was calculated from the date of diagnosis to date of death or loss to follow-up.
Results: Eighteen (18) dogs were enrolled (March 2019 to November 2020). At presentation, 2/18 had confirmed hepatic metastasis. AEs during administration of Immunocidin were infrequent and included hypertension, fever, hypersensitivity and lethargy. One patient also experienced limb and facial twitching and was removed from the study. The most common AEs following treatment included lethargy, hyporexia, and diarrhoea. One patient developed VCOG grade V diarrhoea, thrombocytopenia, and anaemia (cause uncertain). Doxorubicin dosing was reduced to 27 mg/m^2 due to observed gastrointestinal AEs. The median OST was 147 days (range: 39–668 days).
Conclusion: The combination of doxorubicin and Immunocidin appeared to cause more gastrointestinal effects than is expected with doxorubicin alone. No improvement in OST was noted in this small group of dogs.
Funding Information: This study was generously funded by NovaVive.

IMMUNOTHERAPY IN THE TREATMENT OF PATIENTS WITH MELANOMA: ONCOTHERAD NANO-IMMUNOTHERAPY

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Introduction: Canine oral melanoma (COM) is a highly aggressive and metastatic cancer. Many studies demonstrate post-treatment median survival time range from 4.8 to 12 months. Conventional treatments have not shown suitable disease control. A new perspective is represented by Oncotherad is a nanostructured inorganic phosphate complex associated to glycosidic protein, developed by University of Campinas (UNICAMP)/Brazil. Leads to distinct stimulation of the innate immune system mediated by Toll-like receptors TLRs) 2 and 4, resulting in an increased activation of the IFN signalling pathway. Furthermore, decrease the expression of receptor activator of nuclear factor-κB (RANK) and receptor activator of nuclear factor-κB ligand (RANK-L) system, resulting in prevent the formation of metastases and/or counteract their progression.. The aim of the study was to evaluate the efficacy of OncoTherad for first-line chemotherapy-relapsed high-grade COM (with or without metastasis).

Methods: Including 19 animals (8 male, 11 female), median age 12,5 years; OncoTherad treatment (G1 group, $n = 10$) was initiated with twice per week intramuscular (22 mg/ml) application for 3 months, followed by one every other week application until 1 year of treatment. OncoTherad was associated (G2 group, $n = 09$) with chemotherapeutic (Carboplatin: 250 mg/m^2 to 300 mg/m^2—intravenous follow-up 24 months.

Results: Based on RECIST criteria, overall complete response rate was 31.6%, overall partial response rate was 42.1% and overall stable disease rate was 10.5%. Only 15.8% of total patients presented progressive disease. Median overall progression-free survival was 640 days.

Conclusion: In conclusion, OncoTherad seems an effective treatment option for chemotherapy-relapsed COM patients and may provide benefit for preventing tumour progression.

Funding Information: Conselho Nacional de Desenvolvimento Científico e Tecnológico (Cnpq). Fundação de Amparo à Pesquisa do Estado de São Paulo (Fapesp).

INCIDENCE AND MANAGEMENT OF CYTOKINE RELEASE SYNDROME IN VETERINARY MEDICINE

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Introduction: Advances in cancer immunotherapeutics have led to great interest in their potential to treat malignancies. Recently, preliminary and promising results have been reported in dogs with osteosarcoma (OSA). The critical role of T cells in anti-cancer immunity has been demonstrated in various immunotherapies. In this study, 50 dogs with OSA were randomized to receive ECI therapy which combines autologous tumour cell vaccines and adoptive ex vivo activated autologous T-cell transfer. The dogs also received interleukin (IL-2) post T-cells. Toxicities associated with immunotherapies have been reported, including cytokine release syndrome (CRS). Here we review the adverse events (AE) and CRS mitigation plan for dogs undergoing ECI adoptive T cell therapy.

Methods: All dogs underwent amputation surgery to initiate treatment and harvest tissue for autologous vaccine preparation. After administration of three vaccinations, T cells were harvested via apheresis and then ex vivo expanded and activated before being reinfused. Low-dose IL-2 was given post infusion. Dogs were monitored for 4–6 h after infusion.

Results: 33% of dogs experienced a grade 1/2 AE, with no grade 3 or higher AEs reported.

Most common AEs reported were lethargy, pyrexia and nausea.

Three dogs received dexamethasone, per protocol medical management/CRS mitigation plan, with two experiencing grade 2 lethargy and one experiencing grade 2 epistaxis.

We will review the impact of T-cell therapies in humans and dogs, and potential future approaches.

Conclusion: Preliminary safety data from ECI cohort ($n = 50$) in the randomized ECI-OSA-04 study further confirms positive safety profile reported in the previously completed ECI-OSA-01 study ($n = 14$).

Funding Information: ELIAS Animal Health provided funding for the study discussed in the abstract and honoraria to speaker/presenters.

INCREASED TUMOUR INFILTRATING LYMPHOCYTE DENSITY IS ASSOCIATED WITH FAVOURABLE OUTCOMES IN A COMPARATIVE STUDY OF CANINE HISTIOCYTIC SARCOMA

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Introduction: Histiocytic sarcoma (HS) is a rare and aggressive tumour in humans with no universally agreed standard of care therapy. Spontaneous canine HS exhibits increased prevalence in specific breeds, shares key genetic and biologic similarities with the human disease, and occurs in an immunocompetent setting. Previous data alludes to the immunogenicity of this disease in both species highlighting the potential for their successful treatment with immunotherapy.

Methods: Immunohistochemical quantification of CD3 tumour infiltrating lymphocytes (TIL) in five cases of human HS revealed variable intratumoral T cell infiltration. Due to the paucity of human cases and lack of current model systems in which to appraise associations between anti-tumour immunity and treatment-outcome in HS, we analysed clinical data and quantified TIL in 18 dogs diagnosed with localized HS and treated with curative-intent tumour resection with or without adjuvant chemotherapy. Transcriptional analyses of canine HS tumours were performed using the NanoString nCounter Canine IO Panel.

Results: As in humans, assessment of TIL in canine HS biopsy tissues taken at diagnosis reveal a spectrum of immunologically 'cold' to 'hot' tumours. Importantly, we show that increased CD3 and granzyme B TIL are positively associated with favourable outcomes in dogs following surgical resection. Transcriptional analyses confirmed a pro-inflammatory tumour microenvironment in pulmonary HS when compared to splenic HS.

Conclusion: Based on these findings, we propose that spontaneous canine HS is an accessible and powerful novel model to study tumour immunology and will provide a unique platform to appraise the efficacy and tolerability of anti-cancer immunotherapies for HS.

Funding Information: Funding for this study was provided by NCI K08CA252619 (MJA) and internal funds provided to Jennifer A Lenz, Department of Clinical Sciences and Advanced Medicine, School of Veterinary Medicine and Robert G Maki Department of Medicine, Perelman School of Medicine, University of Pennsylvania. The Penn Vet Comparative Pathology Core is supported by the Abramson Cancer Center Support Grant (P30 CA016520). The scanner used for whole slide imaging and the image analysis software was supported by a NIH Shared Instrumentation Grant (S10 OD023465-01A1).

INTESTINAL DYSBIOSIS IN CANINE PATIENTS WITH HEMANGIOSARCOMA

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Introduction: Intestinal dysbiosis is an alteration in the composition and/or richness of the intestinal microbiota. An association between dysbiosis has been hypothesized with cancer pathogenesis, progression and response to therapy. The objective of this study was to determine if canine patients diagnosed with hemangiosarcoma have intestinal dysbiosis, using a qPCR-based dysbiosis index (DI). Determining whether patients with hemangiosarcoma have intestinal dysbiosis is the first step towards understanding whether or not intestinal dysbiosis plays a role in disease development and progression.

Methods: Patients with benign or malignant causes of hemoabdomen secondary to presumed splenic tumour rupture underwent dysbiosis index analysis at the time of splenectomy. A Simon-two-stage design will be used to explore the hypothesized association between dysbiosis and hemangiosarcoma in dogs. In addition, next generation sequencing approaches are under evaluation as an alternative analysis of dysbiosis.

Results: Our preliminary but ongoing analysis suggests a potential association between dysbiosis and a diagnosis of hemangiosarcoma in dogs with splenic tumour rupture. 1/7 dogs with non-neoplastic causes of hemoabdomen had abnormal dysbiosis index. In dogs with splenic hemangiosarcoma 3/10 had an abnormal dysbiosis index. Additional results will continue to be accumulated, analysed, and reported.

Conclusion: Preliminary data suggests an association between intestinal dysbiosis and canine hemangiosarcoma. Additional dogs with benign and malignant splenic tumours are under analysis. If there is an association between hemangiosarcoma and dysbiosis we expect future questions regarding the role of dysbiosis on patient prognosis, disease progression and therapy.

Funding Information: Funding through Ethos Discovery.

INVESTIGATING HISTOTRIPSY AS A NOVEL TREATMENT FOR CANINE OSTEOSARCOMA

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Introduction: The aim of this study was to demonstrate the feasibility of treating canine osteosarcoma (OS) with histotripsy, a novel non-thermal focused ultrasound tumour ablation technique. This study evaluated the ablative effect of histotripsy on canine OS cells and on the immune profile of peripheral blood mononuclear cells. Evaluation of the ablative and immune effects of histotripsy in OS informs

development of histotripsy as a non-invasive limb salvage and immunotherapeutic for OS.

Methods: Five canine OS patients were enrolled in a clinical trial. The primary tumour was treated with histotripsy and a standard-of-care limb amputation was performed 1 day after histotripsy treatment. Histotripsy treatment was performed using a 500 kHz transducer with an ultrasound imaging probe coaxially aligned for real-time image guidance and monitoring of cavitation bubble cloud formation. A 2-cm spherical portion of the tumour was treated with histotripsy at a pulse repetition frequency of 500 Hz and a dose of 500 pulses/point. Treated tumours were evaluated grossly and histologically after surgical excision. Pre- and post-histotripsy treatment peripheral blood samples were collected from three dogs for immune cell phenotyping via flow cytometry.

Results: Tumour ablation zones were successfully identified grossly and microscopically, ablated tumour volumes corresponded with planned treatment volumes and effective ablation of tumour cells was noted microscopically. Immune cell phenotyping identified an increase in peripheral CD4+ T lymphocytes post-histotripsy treatment.

Conclusion: Histotripsy successfully ablated the targeted area within the primary tumour, demonstrating its potential to serve as a novel treatment modality for OS.

Funding Information: 1. American Kennel Club Canine Health Foundation.

LINE-1 METHYLATION ANALYSIS AS A LIQUID BIOPSY BIOMARKER FOR CANINE SPLENIC HEMANGIOSARCOMA

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Introduction: DNA methylation is known to play an important role in cancer development. The long interspersed nuclear element 1 (LINE-1) is a repetitive sequence interspersed throughout the genome of some organisms. LINE-1 hypomethylation has been reported in human malignancies, it is used as an epigenetic cancer biomarker. This study aimed to determine the diagnostic value of LINE-1 methylation in dogs with splenic masses using tissue and cell free DNA samples.

Methods: Genomic DNA was isolated from splenic masses in 12 dogs with hemangiosarcoma, nine dogs with other malignant tumour, and 13 dogs with benign tumours. LINE-1 methylation was measured using methylation sensitive and insensitive restriction enzyme digestion, followed by real-time PCR. Additionally, blood samples were collected from some patients to isolate cell free DNA for determining LINE-1 methylation status throughout the course of disease progression.

Results: LINE-1 methylation in tumour samples was significantly lower in patients with hemangiosarcoma than in those with other malignant and benign tumours groups. Similar results were observed in cell free DNA samples. There was a measurable correlation between methylation level and clinical status during follow-up in some cases.

Conclusion: These results indicate that LINE-1 methylation may be a predictive biomarker for splenic hemangiosarcoma in dogs. Additionally, cell free DNA methylation analysis may be a potentially useful tool for monitoring the progression of splenic malignancies. Further research is required to establish the true clinical value of LINE-1 methylation status in cell free DNA as a biomarker for canine splenic hemangiosarcoma.

Funding Information: This study was supported by THE AKIYAMA LIFE SCIENCE FOUNDATION.

NIMUSTINE (ACNU) TREATMENT FOR REFRACTORY HIGH-GRADE MULTICENTRIC LYMPHOMA IN DOGS

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Introduction: Nimustine (ACNU) is an alkylating agent that belongs to nitrosourea as lomustine (CCNU). To date, few studies have examined the usefulness of ACNU treatment for canine lymphoma. The objective of this retrospective study was to evaluate adverse events and efficacies of ACNU treatment as rescue therapy for canine refractory high-grade multicentric lymphoma.

Methods: This study included dogs with high-grade multicentric lymphoma that were refractory to CHOP protocol and treated by ACNU, and medical records of these dogs were retrospectively reviewed.

Results: Fourteen dogs were included in this study. The median starting dosage of ACNU was 23 mg/m^2 (range, 20–25 mg/m^2) with dosing intervals of 3 weeks. Three dogs (21%) developed grade 4 neutropenia, two dogs (14%) developed grade 4 thrombocytopenia, and three dogs (21%) showed grade 3 ALT elevation. No significant gastrointestinal toxicity and febrile neutropenia were observed. Nine of the 14 dogs were eligible for assessment of the response to ACNU treatment, and three, four and two dogs achieved CR, SD and PD,

respectively. The median overall survival after administration of ACNU was 88 days (range, 11 to >626 days), and the median progression-free survival was 7 days (range, 0 to >626 days).

Conclusion: ACNU treatment was well tolerated in dogs with high-grade multicentric lymphoma refractory to CHOP protocol. The response rates and remission durations in this study were comparable to those in other rescue protocols, but further studies using larger number of dogs are needed to investigate the efficacies of ACNU treatment.

Funding Information: No conflicts of interest have been declared. None of the authors of this study has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of this study.

PROSPECTIVE EVALUATION OF THE FAECAL MICROBIOME IN DOGS WITH LYMPHOMA TREATED WITH CHOP CHEMOTHERAPY

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Introduction: Faecal microbiome composition may modulate efficacy of cancer therapy and risk of side effects in humans. Some chemotherapy agents may lead to reduced gut microbial diversity in humans. The relationship between microbiota and chemotherapy efficacy and tolerability has not been investigated in dogs. We aimed to evaluate (1) changes in faecal microbial diversity during a cycle of CHOP and (2) whether these changes correlated with adverse events or treatment response.

Methods: Eighteen dogs with lymphoma were prospectively enrolled, and stool samples were collected weekly for 6 weeks during CHOP. Faecal microbiome was analysed by 16S rRNA amplicon sequencing using an established protocol. Treatment-associated differences in richness, alpha and beta diversity were determined by comparison to data from healthy controls ($n = 27$) using factorial ANOVA and PERMANOVA.

Results: Dogs with lymphoma had decreased faecal microbial diversity when compared with healthy controls at baseline ($p = .0002$) and throughout treatment ($p = .0002, .0039, .0001$). Alpha and beta diversity did not significantly change in dogs throughout a cycle of CHOP chemotherapy ($p = .284$ and $.93$, respectively). Samples pre-treated with antibiotics were less diverse (alpha and beta diversity) than untreated samples ($p = .001, .0001$). Some treated dogs developed an increased abundance of Proteobacteria during treatment.

Conclusion: The faecal microbiome of healthy dogs and dogs with lymphoma receiving CHOP is relatively stable over time, but dogs with lymphoma have reduced microbial diversity compared to healthy dogs. An increase in Proteobacteria abundance during treatment may be related to chemotherapy or antibiotic use.

RETROSPECTIVE EVALUATION OF THE EFFECT OF A WHOLE CELL ADJUVANTED AUTOLOGOUS CANCER VACCINE ON THE POSTOPERATIVE SURVIVAL OF DOGS WITH HIGH-RISK MAST CELL TUMOURS

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Introduction: High-grade mast cell tumours (MCT) have a reported post-operative median survival time of 3.6 months with 100% mortality by 600 days in dogsm (1). Adjuvant chemotherapy is therefore recommended. Immunotherapy has not been extensively studied in dogs with MCT. The purpose of this retrospective study was to determine the postoperative survival in dogs with high-risk MCT treated only with Torigen's autologous cancer vaccine (ACV).

Methods: Torigen's database was queried to identify dogs with MCT. Dogs were eligible for inclusion if they had histopathologic diagnosis of high-grade cutaneous MCT, or evidence of metastasis regardless of histologic grade, and received at least one dose of the ACV with no other therapy.

Results: Fourteen dogs qualified for study. Mean age of the dogs was 10.0 years; mean weight was 23.0 kg. Overall median survival time was 194 days (range, 22–813 days). Five dogs (36%) were right-censored because they were still alive at the time of analysis (range, 457–813 days). The median survival time of dogs without metastasis (277 days; $n = 8$) was not significantly different ($p = .4860$) than dogs with metastasis (134 days; $n = 6$). Three dogs experienced mild, self-limiting adverse events including lethargy, anorexia, and injection site swelling.

Conclusion: The ACV was well-tolerated by this group of dogs. Over 1/3 of these high-risk MCT patients had prolonged survival times when treated with adjuvant immunotherapy. These preliminary findings warrant prospective evaluation of Torigen's ACV for canine MCT.

Funding Information: No outside funding for this work.

ROLE OF PI3K PATHWAY IN REPROGRAMMING THE TUMOUR NICHE OF CANINE HEMANGIOSARCOMA

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Introduction: Hemangiosarcomas (HSAs) are aggressive, highly metastatic cancers that form malignant vessels. This tumour is more prevalent in dogs than any other animals, and outcomes for affected dogs are generally unfavourable. Recurrent mutations in PIK3CA gene were observed in canine HSAs, and they appear to establish angiogenic programs. In this study, we hypothesize that PIK3CA mutations activating PI3K pathway contribute to the molecular programs that create the immune niche in canine HSA.

Methods: We first engineered canine HSA cells to induce PIK3CA H1047R mutations using CRISPR/Cas9, followed by clonal selection of mutant and wild-type (WT) cells. Then, we examined cell growth rate and expression of gH2AX, DNA damage marker in mutant and WT cells with treatment of PI3K-alpha selective inhibitor (BYL719) and pan-PI3K inhibitor (ZSTK474).

Results: Our results showed that BYL719 decreased cell proliferation of PIK3CA mutant cells by 40–50% at 10uM compared with that of WT cells, while no significant difference was observed between mutant and WT cells treated with ZSTK474. Expression levels of gH2AX were increased in both mutant and WT cells with treatment of BYL719 and ZSTK474; however, the ratio of change in gH2AX expression was smaller in mutant cells than that in WT cells.

Conclusion: These data suggest that activation of PI3K pathway is essential for tumour cell growth and that PIK3CA mutation-induced pathways are targetable by PI3K-alpha selective inhibitor in canine HSA. Our ongoing work is to determine if canine HSA cells have cell-autonomous capacity to govern haematopoietic progenitors and immune cells, potentially establishing the tumour immune niche.

Funding Information: This work is supported by grant (#02759) from the AKC Canine Health Foundation and by the Animal Cancer Care and Research Program of the University of Minnesota.

SHIP INHIBITION IN CANINE OSTEOSARCOMA: EVALUATION OF A NOVEL TARGET

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Introduction: Despite advances in the understanding of cancers across species, the treatment paradigm for both human and canine osteosarcoma (OS) has not changed in decades. SHIP proteins, important phosphatases in the PI3K cell signalling tree, have been shown to promote cell survival in various neoplasms, but their role in canine OS has not been evaluated. Therefore, we sought to determine the in vitro biologic effects of several novel SHIP inhibitors.

Methods: MTS experiments assessed cell viability in three established canine OS lines (Abrams, D-17 and HMPOS). Cells were treated with 3-AC, K118, K149 and K161 at concentrations ranging from 1.25 to 10.0 μM. To assess cell migration, confluent canine OS cells were wounded using BioTek's Autoscratch Tool followed by treatment with K118. Wells were imaged for 72 h post-treatment using the Lionheart FX.

Results: MTS data demonstrated that SHIP inhibitors exhibit a time and concentration dependent decrease in cell viability. The greatest effect for all inhibitors occurred 72 h post-treatment. K118 was chosen for further evaluation as it demonstrated the highest potency of SHIP inhibitors tested. K118 inhibited wound healing and cell migration; its greatest effects were noted at 1.25 and 2.5 μM concentrations.

Conclusion: Further studies of SHIP inhibitors should investigate the mechanism of cell signalling pathway interference, specifically in the PI3K/mTOR pathway. Clarifying the role of SHIP proteins in OS pathogenesis could promote further in vivo studies, assessing biologic efficacy.

Funding Information: This work was funded by extramural (Morris Animal Foundation Veterinary Scholar Program) and intramural (Phi Zeta Research Grant Proposal–University of Missouri College of Veterinary Medicine) grants. Ms. Allen received a funded Boehringer-Ingelheim Veterinary Student Research Award for this work.

TEMPORAL CHARACTERIZATION OF BLOOD–BRAIN BARRIER DISRUPTION INDUCED BY HIGH-FREQUENCY IRREVERSIBLE ELECTROPORATION (H-FIRE) TREATMENT

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Introduction: Glioblastoma (GBM) is the most common and deadliest of malignant primary brain tumours affecting humans. Extensive local invasion by the primary tumour and its location behind the blood brain barrier (BBB) prevents adequate delivery of most chemotherapy agents. H-FIRE represents a novel non-thermal ablation method for safe and potentially effective treatment of GBM. H-FIRE transiently disrupts the BBB, allowing for electrochemoablation of microscopic tumour cells located within the tumour penumbra. Here, we characterize the mechanisms of H-FIRE mediated BBB disruption (BBBD) in normal rat brain.

Methods: Intracranial H-FIRE was delivered to Fischer rats prior to sacrifice at predetermined time points. Western blotting and immunoprecipitation were performed on brain lysates to detect tight junction

(TJ) proteins in their native and ubiquitinated forms, and cytoskeletal proteins. TJ proteins were also evaluated with immunofluorescence. RNA isolates from regions of BBBD were pooled for gene expression and pathway analysis, completed using commercially available SuperArray plates containing genes associated with TJs. Data was analysed using Qiagen Ingenuity Pathway Analysis (IPA) software. Significant results were confirmed using qRT-PCR.

Results: TJ protein expression decreased following H-FIRE, with gradual recovery over time. Increased ubiquitination of claudin-5 and occludin was apparent 1–48 h post-H-FIRE compared to controls. F-actin/total actin and F/G actin ratios were significantly decreased 1-hour post-H-FIRE compared to all other treatment groups. An increase in ZO-1 and claudin-5 gene expression was observed over time relative to controls, consistent with BBB recovery. Relative expression of occludin was decreased at all time points. IPA analysis revealed significant dysregulation of claudin genes, centred around claudin-6 and peaking at 72 h post-H-FIRE, which was further supported by qRT-PCR.

Conclusion: In conclusion, H-FIRE transiently permeates the BBB via cytoskeletal remodelling, altered TJ gene expression and subsequent protein assembly, and increased TJ protein degradation.

Funding Information: Internal Research Competition Grant.
RO1CA213423, NIH/NCI.
PO1CA207206, NIH/NCI.