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2016 ACVIM Forum Research Report Program

2016 ACVIM Forum Research Report Program
Denver, Colorado, June 9 - 11, 2016
Index of Abstracts

Thursday, June 9

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2:35 pm	Luis Gaitero	Micrornas MIR-21 and MIR-181C in Cerebrospinal Fluid and Serum in Canine Meningoencephalomyelitis of Unknown Origin
3:10 pm	William Bush	C-Reactive Protein in the Diagnosis of Discospondylitis
3:35 pm	Melissa Lewis	Do Dogs Spinal Walk? Electrophysiologic Characterization of Long Tract Integrity in Canine Spinal Cord Injury
4:25 pm	Andrea Tipold	Does a Th17 Skewed Immune Response in Steroid-Responsive Meningitis-Arteritis Exist?
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**Also See Small Animal Internal Medicine abstracts, Saturday, June 11.

9:25 am	George Lubas	Electrochemotherapy with Intravenous Bleomycin for Treatment of Feline Squamous Cell Carcinoma: Experience on 12 Cats
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10:55 am	Shay Bracha	Osteosarcoma-Derived Exosomes Impair CD4+ and CD8+ T-Cell Proliferation and Induce T-Regulatory Cell Expansion

SMALL ANIMAL INTERNAL MEDICINE

8:00 am	Eva Furrow	Three Diverse Mutations Underlying Canine Xanthine Urolithiasis
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11:30 am	Allyson Berent	Subcutaneous Ureteral Bypass Device Placement for Benign Ureteral Obstruction in Cats: 137 Cats (174 Ureters)
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4:25 pm	Hannes Lohi	Prevalence of Genetic Disease Variants in 100,000 Purebred and Mixed Breed Dogs
4:50 pm	Sharon Center	Aminoaciduria May Explain Hypoaminoacidemia in Canine Hepatocutaneous Syndrome (n=20)

EQUINE

8:00 am	Mark Bowen	The Assessment of Behavioral Changes Displayed in Horses with Equine Glandular Gastric Disease
8:25 am	Ben Sykes	Pharmacodynamics of a Long-Acting Injectable Formulation of Omeprazole in the Horse
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9:25 am	Kathleen Ivester	Immunoproteomic Analysis of Inhalable Barn Dust
10:30 am	Kelsey Hart	Effects of Free and Carrier-Bound Cortisol on Equine Neutrophil Function
10:55 am	M. Julia Felippe	Bone Marrow Transplantation and Epigenetic Modulation Of Hematopoietic Precursors in Equine Common Variable Immunodeficiency
11:30 am	Amy Johnson	Serum and CSF Lyme Multiplex Results for Neurologic Horses with and without Neuroborreliosis
11:55 am	Adam Krull	Use of Enrichment and Quantitative PCR to Improve Detection of Salmonella in Referral Hospitals

this study was to compare concentrations of serum IGF-1, insulin, glucose, ketones, and lactate concentrations in dogs with lymphoma compared with age, sex, and weight-matched controls. Dogs with naïve lymphoma (n = 16) were identified and matched with controls (n = 16) on the basis of age, sex, and weight. IGF-1, insulin, glucose, ketones, and lactate concentrations were measured in fasted blood samples. Variables were compared between groups and analysed for correlations between the individual variables. Insulin, IGF-1 and glucose were not different between cases and controls. However, lactate and ketones were higher in cases than in controls ($P = 0.007$, $P < 0.001$) and these variables were correlated with each other (r = 0.450, $P = 0.011$). Insulin and IGF-1 (r = 0.457, $P = 0.009$) were also correlated across both groups of dogs. This study did not demonstrate increased IGF-1 and insulin in this small group of dogs with lymphoma compared to the controls. However, ketones were higher in the cases and correlated with lactate, which suggests that they could also be a useful biomarker of metabolism in dogs with lymphoma.

ELECTROCHEMOTHERAPY WITH INTRAVENOUS BLEOMYCIN FOR TREATMENT OF FELINE SQUAMOUS CELL CARCINOMA: EXPERIENCE ON 12 CATS. Alessio Pierini[1], Ron Lowe[2], Valentina Granziera[2], Veronica Marchetti[1], George Lubas[1]. [1]Department of Veterinary Sciences, University of Pisa, Pisa, Italy, [2]Ashleigh Veterinary Clinic Limited, Knaresborough, UK

The aim of this study was to evaluate the efficacy and safety of neoadjuvant electrochemotherapy (ECT) with intravenous bleomycin for the treatment of skin squamous cell carcinoma (SCC) of the head in cats.

Twelve client-owned cats with histological diagnosis of SCC of the head were enrolled. The owners elected to carry out ECT as an alternative treatment to surgery. All cats were staged by physical examination, fine-needle aspiration of mandibular lymph node (if palpable) and three-view thoracic X-rays. Complete blood cell count, serum biochemistry profile and urinalysis also were performed as the patients underwent general anesthesia as well. Cats with lymph node involvement or radiographic features of pulmonary metastases were excluded from this study. Clinical T stage was assigned on the basis of WHO criteria. The longest tumor diameter based on physical examination was used for clinical staging and follow-up.

For ECT treatment, cats were premedicated with medetomidine and butorphanol. Once sedated, an IV catheter was placed in a peripheral vein, cats were preoxygenated via a face mask, and anesthesia was induced with alfaxalone administered IV. Then cats were intubated and anesthesia was maintained with oxygen, nitrous oxide and isoflurane. Bleomycin was diluted in three milliliters of saline solution and administered IV at a dosage of 15–20 mg/m^2.

The electroporation was performed using a Cytopulse PA4000 (Cyto Pulse Sciences, Inc) in nine cats or a Cytopulse Oncovet in two cats with Gehl needle electrodes of 1 cm length from the same manufacturer. The pulse pattern employed was 8 monophasic square pulses of 100 microseconds each at a frequency of 1 Hz (interpulse interval 0.9999 sec) with the PA4000 or 5 kHz (interpulse interval 0.01 sec) with the Oncovet. The latter equipment allowed more rapid application of the therapy because of the higher pulse frequency and the improved user interface. The Gehl electrode pattern consists of two parallel 1–1.5 cm rows of 6 needles, the rows being 6 mm apart (Gehl and others 1999). The pulse amplitude was 600–720 volts (1000–1200 volts/cm). This pulse pattern was established by Gehl and others (1999). In one case (periocular SCC) a Cliniporator (IGEA S.p.a.) with 8-needle electrodes and 20 mm length needles were used. The voltage setting for the Cliniporator was pre-set by the manufacturer at 1000 volts/cm with current varying between applications depending on tissue conductivity (safety limit of 20A).

Patients were assessed every 3–10 days for the initial 4 weeks after treatment. Tumor response was assessed every 6–8 weeks following the response evaluation criteria for solid tumours established by veterinary cooperative oncology group consensus document by physical examination, fine-needle aspiration of mandibular lymph node if palpable and 3-views thoracic X-rays. Tumor measurements were made until remission or until relapse occurred. A complete remission (CR) was defined as total reduction of the tumor. A partial remission (PR) was defined as ≥30% reduction in tumor diameter. Stable disease (SD) was defined as <30% reduction in tumor diameter or <20% increase in tumor diameter, and progressive disease (PD) was defined as ≥20% increase in tumor diameter. Presence of new lesions near to the primary tumor or presences of metastasis were considered as PD. A minimum duration of two to three weeks was required for a response to qualify as positive.

Progression-free survival (PFS), median survival time (MST) and overall response rate (ORR) were calculated. PFS was defined as time from ECT until tumor progression, recurrence or death. ST was defined as time from ECT until death. ORR was defined as proportion of cats that reached CR or PR.

The toxicity treatment (especially locally) was assessed by interviewing the owner and carrying out a physical examination up to 7–14 days after ECT. Only early adverse effects were evaluated and a subjective scoring system from zero (no adverse effect) to 4 (toxicity-related death) was used. For each cat, the highest toxicity score was used for analysis.

Eleven of twelve SCC were localized on the nasal planum. One was localized on the periocular region. Ten were neutered males and 2 were spayed females. Ages ranged from 7 to 16 years old (median, 11.4). SCCs were classified with WHO T-stage as T1 (#7), T2 (#3), T3 and T4 (one each).

Sixteen ECT treatments were done. The cat with periocular SCC had four treatments and one cat with nasal planum SCC had two treatments. Eleven of 12 cats were eligible for survival analysis. A cat with nasal planum T1 SCC due to treatment toxicity (toxicity score = 4) at 9 days after ECT was euthanized.

For survivor cats, ORR was 100%. There were 8 (73%) CR and 3 (27%) PR. Six (100%) of 6 cats with T1 SCC had CR and two cats (66%) of 3 T2 SCC had CR. One cat with T2 and two cats with T3 and T4 SCC had PR. All cats were dead at the end of the study. Three cats developed PD, with a median PFS of 253 days (range, 97–468 days). All of them had PR. One cat with a nasal planumT3 SCC developed recurrence after 97 days after the first ECT treatment and was treated once again. This cat was euthanized for PD 231 days after the first ECT. One cat had a periocular T2 SCC developed the first recurrence 468 days after ECT. Overall, this cat was treated for a total of four times for new recurrences and was euthanized 730 days after the first ECT. The third cat had a nasal planum T4 SCC and was euthanized 194 days after ECT. The other eight cats were died for unrelated causes. No cats developed metastasis. Overall MST was 452 days (range, 194–2973 days). MST for cats that achieved CR was 452 days (range, 194–2973 days) and 231 days (range, 194–730 days) for cats that achieved PR. MST was calculated for any T stage and was 397 days for T1 (range, 251–2973), 730 days for T2 (range, 599–1731), 231 days for T3 and 194 days for T4.

All twelve cats were evaluated for toxicity. All cats developed early effects. Toxicity was classified as grade 1 (#8), grade 2 (#1), grade 3 (#2) and grade 4 (1). Toxicity was mild in almost all T1 SCC (6 grade 1, 1 grade 4).

Several therapeutical strategies are described to treat feline SCC of the head such as surgery, external-beam radiation therapy, strontium-90 plesiotherapy, cryosurgery, photodynamic therapy, laser application, hyperthermia, thermocautery with curettage and electrochemotherapy. However, only superficial cancers can be managed effectively with almost the treatments described above.

Nowadays, ECT has become a reliable treatment for cutaneous SCC of the head in cats. Recently, two papers evaluated the efficacy of ECT delivered five-minute after intravenous bleomycin in feline cutaneous SCC at varies WHO T stage. Tozon et al. (2014) observed an ORR of 87.5% for sixteen superficial SCC in cats. Spugnini et al. (2015) reported the capability of ECT to improve bleomycin efficacy for feline SCC. Twenty-six cats were treated with intravenous bleomycin coupled with ECT and 21 cats were treated with bleomycin alone. ORR in the ECT treated group was 88.5% versus 33% for control group. Median time to progression in the ECT treated animals was 30.5 months, whereas in controls it was 3.9 months. In the present study an overall response rate of 100% and a MST of 452 days (about 15 months) were observed. Many reports have indicated that aggressive local tumor control offers the best chance to obtaining clinical tumor response and long survival times. However, tumor control achieved by aggressive surgical excision or external beam radiation therapy is dependent on reaching adequate surgical margins or the degree of response to radiation therapy. In a previously study, cats with SCC treated with ECT that did not reach CR were more likely to

be in an advanced WHO T stage. In the present cohort of cats PR was reached in T3 and T4 SCC and in 4-cm T2 SCC. Authors speculated that more advanced T stage SCCs can benefit of more ECT treatments until CR is reached or can eligible to have a multimodal therapy.

Moreover, aggressive surgical excision can be limited by anatomical localization and cosmetic concerns for owners. On the other hand, radiation therapy is limited by early and late effects on the eyes in periocular SCC and by financial concerns for owners. In the present study early effects were considered well tolerated in nine of twelve cats. Two cats with severe early effects had a T4 SCC and a 4-cm periocular SCC. Severe effects can occur more frequently in cats with bulky or infiltrative tumor. Discharge instructions for owners advised application of an Elizabethan collar on cats. Nevertheless, the present study did not review symptomatic therapeutic modalities that authors used in management of pain and/or scratching. Non-steroidal antinflammatory drug such as meloxicam was previously reported for successfully early effect management in ECT treated cats. Furthermore in the present study one cat with a T1 SCC developed an ECT toxicity-related death. The major limit to interpret toxicity-related death is that cat was euthanized due to owner's decision and authors were not able to confirm whether the owner followed discharge instructions for scratching control.

This study has some limitations. First, the small number of enrolled cats can influence ORR, MST and toxicity evaluations. Second, the subjective evaluation of toxicity makes interpretation of these data difficult. Finally, ORR and MST can be influenced by the use of three different electroporation machines and a non-standard dose of intravenous bleomycin.

In conclusion, intravenous bleomycin coupled with ECT is well tolerated for cutaneous SCC in cats. The results of this study suggest that ECT should be considered as an alternative treatment option, especially in superficial SCC, and when owners do not accept other treatment approaches due to cosmetic or financial concerns.

Gehl J, Sorensen TH, Nielsen K, Raskmark P, Nielsen SL, Skovsgaard T, et al. In vivo electroporation of skeletal muscle: threshold, efficacy and relation to electric field distribution. Biochimica et Biophysica Acta 1999; 1428: 233–240.

Spugnini EP, Pizzuto M, Filipponi M, Romani L, Vincenzi B, Menicagli F, et al. Electroporation Enhances Bleomycin Efficacy in Cats with Periocular Carcinoma and Advanced Squamous Cell Carcinoma of the Head. J Vet Intern Med 2015;29(5):1368–75.

Tozon N, Pavlin D, Sersa G, Dolinsek T, Cemazar M. Electrochemotherapy with intravenous bleomycin injection: an observational study in superficial squamous cell carcinoma in cats. J Feline Med Surg. 2014 Apr;16(4):291–9.

NOVEL ONCOLYTIC MARABA VIRUS FOR THE ADJUVANT TREATMENT OF FELINE MAMMARY CARCINOMA. J. Paul Woods[1], Byram Bridle[2], Michelle Oblak[1], Robert Foster[2], Victoria Sabine[1], Jeff Hummel[3], Brian Lichty[3]. [1]Clinical Studies, Ontario Veterinary College, University of Guelph,, Guelph, ON, Canada, [2]Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, [3]McMaster Immunology Research Centre, McMaster University, Hamilton, ON, Canada

Feline mammary carcinomas (FMC) are highly metastatic tumours frequently (>80%) displaying metastasis to the regional lymph nodes, lungs, pleura, and liver. Historically, the treatment of FMC has been surgery; however, at the time of diagnosis, FMC is often in an advanced stage due to its rapid progression. Following surgery, the median disease free survival reported is less than a year with a 2-year survival rate of <20%. Adjuvant chemotherapy (e.g. doxorubicin) has not shown benefit in delaying relapse or prolonging life with FMC.

Oncolytic viruses are viruses whose replication is restricted to tumour cells leading to cytolysis. A growing number of viruses are being developed as oncolytic viruses and various strategies have been utilized to target tumour cells. Our group developed a biotherapeutic that combines two emerging cancer treatment modalities: tumour vaccination and oncolytic viruses. We recently determined that oncolytic viruses expressing tumour antigen transgenes are an excellent means to boost and enhance tumour vaccination.

Therefore, we are conducting a clinical trial combining the two emerging cancer treatment modalities, tumour vaccination and oncolytic viruses, to treat FMC following surgery (i.e. adjuvant therapy in addition to surgical excision). Our goal is to test our heterologous prime:boost strategy targeting tumour antigens relevant to FMC. We hypothesize that induction of anti-tumoural immunity and direct oncolysis of metastatic (often occult) disease using our oncolytic vaccine booster will delay or prevent post-surgical relapse and extend survival in cats with mammary carcinoma.

Cats with FMC (histologically confirmed) were staged (CBC, biochemical profile, thoracic radiographs, abdominal ultrasound or CT). Cats were vaccinated with an adenovirus expressing a cancer gene, then 2 weeks later the tumour was excised (radical mastectomy), and then 4 weeks postoperatively the cats received a booster with an oncolytic virus (Maraba virus) also expressing the cancer gene. Cats were followed up with physical exams and thoracic radiographs.

Twelve female (spayed) cats have entered the study with a mean age of 12.5 years (range 7.5–16.8), median weight of 4.4 kg (range 3.25–5.89), consisting of 7 DSH, 4 DLH, 1 Turkish Van. The FMC consisted of 7 stage 1, 1 stage 2, and 4 stage 3. During oncolytic virus infusion 1 cat died of putative anaphylaxis (severe acute diffuse pulmonary hemorrhage and edema on post mortem). Otherwise toxicity of the adenovirus vaccine, surgery and marabavirus infusion was manageable. Following the anaphylactic death, pre-infusion skin testing of the marabavirus was introduced for the 5 subsequent cats and all 5 cats were negative. Six cats are dead with 5 dying of disease and 1 dying due to putative anaphylaxis during the oncolytic virus infusion treatment. Six cats are still alive (ranging from 92–568 days). The median overall survival is 240 days.

Except for the one catastrophic anaphylactic reaction, the treatment is well tolerated. Employing a novel oncolytic virus to implement the heterologous prime:boost strategy to target tumour antigens may result in a new modality for the adjuvant treatment of FMC following surgical excision.

OSTEOSARCOMA-DERIVED EXOSOMES IMPAIR CD4+ AND CD8 + T-CELL PROLIFERATION AND INDUCE T-REGULATORY CELL EXPANSION. Shay Bracha[1], Liping Yang[3], Claudia Maier[3], Cheri Goodall[1], Ryan Troyer[2]. [1]Oregon State University-Department of Clinical Sciences, Corvallis, Oregon, USA, [2]Oregon State University-Department of Biomedical Sciences, Corvallis, Oregon, USA, [3]Oregon State University-Department of Chemistry, Corvallis, Oregon, USA

Exosomes are microvesicles secreted by cells that function in cell-to-cell communication through cellular uptake of exosomal protein and RNA cargo, as well as interaction with surface-expressed proteins. Exosomes are crucial for tumor progression, angiogenesis and immune evasion. Although the role of tumor exosomes has been described in numerous studies, the effect of osteosarcoma exosomes on T-cells is lacking. We hypothesize that osteosarcoma-derived exosomes carry a vastly different cargo in comparison to exosomes from healthy osteoblasts, and include immunosuppressive proteins which directly impact healthy T-cells by reducing the proliferation and activation of CD4 + and CD8 + cells and enhancing the expansion of T-regulatory cells. Exosomes were isolated from the media of osteosarcoma (HmPos) and healthy canine osteoblast (CnOb) cells (Total Exosome Isolation, Invitrogen). Exosome size and concentration was validated by NanoCyte single molecule imaging. Exosomal proteins were digested and subjected to mass spectrometry (LC-MS/MS), followed by data analysis using MASCOT algorithm and Scaffold software. Additionally, intact exosomes were co-incubated with lymphocytes from a healthy dog for 3 and 5 days; controls included unexposed lymphocytes and lymphocytes exposed to an equal concentration of exosomes from normal osteoblasts (CnOb). Flow cytometry analyses were performed to measure expression of T cell markers (CD4, CD8, CD25, and Foxp3), cell viability, and cell proliferation. HmPos and CnOb exosomes shared 55 proteins, while 43 and 62 proteins were unique, respectively. HmPos exosomes included proteins which are known to induce immune modulation and evasion (Tenascin, alpha-fetoprotein). T-cells incubated with malignant exosomes exhibited a profound reduction in proliferation and activation of CD4 + and CD8 + T cells at days 3 and 5 ($P < 0.001$). Expansion of the T-regulatory population in the malignant exosome-treated group was significant at day 5 ($P < 0.001$) in comparison to normal osteoblasts. Our study