Received: 24 June 2020 | Revised: 27 July 2020 | Accepted: 27 July 2020

DOI: 10.1111/vco.12642

ORIGINAL ARTICLE

Veterinary and Comparative Oncology WILEY

Safety evaluation of the canine osteosarcoma vaccine, live *Listeria* vector

Margaret L. Musser[1] | Erika P. Berger[1] | Chelsea D. Tripp[2] | Craig A. Clifford[3] | Philip J. Bergman[4,5] | Chad M. Johannes[1]

[1]College of Veterinary Medicine, Veterinary Clinical Sciences, Iowa State University, Ames, Iowa

[2]Oncology Service, Bridge Animal Referral Center, Edmonds, Washington

[3]Oncology Service, Hope Veterinary Specialists, Malvern, Pennsylvania

[4]VCA Katonah Bedford Veterinary Center, Clinical Studies and Oncology Service, Bedford Hills, New York

[5]Adjunct Faculty Member, Memorial Sloan-Kettering Cancer Center, New York, New York

Correspondence
Margaret L. Musser, College of Veterinary Medicine, Veterinary Clinical Sciences, Iowa State University, Ames, IA, USA.
Email: mmusser@iastate.edu

Abstract

Canine osteosarcoma (OSA) is an aggressive bone tumour in dogs. Standard-of-care treatment typically results in relatively short survival times; thus, alternative treatments are needed to confer a survival advantage. It has been shown that OSA is an immunogenic tumour, suggesting that immune modulation may result in superior outcomes. A cryopreserved, *Listeria*-based OSA vaccine was recently developed and an initial study in dogs reported prolonged survival for patients receiving the vaccine in conjunction with standard-of-care. The goal of the current observational study was to report on the safety of the lyophilized formulation of this vaccine (the canine OSA vaccine, live *Listeria* vector [COV-LLV]) in a group of dogs previously diagnosed with OSA. Forty-nine (49) dogs received the COV-LLV and were included for analysis. Adverse events (AEs) noted during and after vaccinations were recorded. The AEs observed were typically mild and self-limiting, with nausea, lethargy and fever being most common. Four dogs (8%) cultured positive for *Listeria* (three infections including an amputation site abscess, septic stifle joint and bacterial cystitis; and one dog whose lungs cultured *Listeria*-positive on necropsy within 24 hours of COV-LLV administration). These cases join the previously reported *Listeria*-positive thoracic abscess that developed in a canine following use of COV-LLV. Although uncommon, it is important to realize this clinically significant AE is possible in patients treated with live therapeutic *Listeria* vaccines. As *Listeria* is zoonotic, caution is required not only for the patient receiving the vaccine, but also for the health care workers and family caring for the patient.

KEYWORDS

cancer vaccines, dogs, immunotherapy, *Listeria monocytogenes*, listeriosis

1 | INTRODUCTION

Canine osteosarcoma (OSA) is the most common primary skeletal tumour in the dog.[1] Standard-of-care treatment includes local disease control with an amputation, limb-salvage procedure, or stereotactic radiation therapy and adjuvant chemotherapy.[2] Despite this relatively aggressive treatment approach, survival times are limited (median 9-11 months, depending on

chemotherapy protocol elected).[2] Thus, alternative treatment approaches are needed.

Since the 1890s, OSA has been recognized as a highly immunogenic tumour, responding positively to a stimulated immune system following inoculation with "Coley's Toxins" derived from *Streptococcus pyogenes*.[3] In both humans and dogs, it has been observed that patients with OSA who underwent limb-salvage surgery and developed an infection of the surgical site were less likely to develop metastatic disease or die due to their OSA compared to patients without an infection.[4,5] In dogs, this resulted in a significant improvement in median survival time (18 versus 7.6 months, respectively).[4] A second canine study corroborated these findings: dogs with OSA undergoing either endoprosthetic limb-salvage therapy or cortical allograft that developed a post-operative infection had a significantly longer survival time (22.8 versus 9.6 months).[6]

Following these observations, many techniques to harness the immune system have been applied to OSA patients in an attempt to improve overall survival time, with varying success. These include injectable Bacillus Calmette-Guérin immunotherapy,[7] liposomal muramyl tripeptide phosphatidylethanolamine,[8] interleukin-2 cytokine therapy,[9] Fas ligand gene therapy,[10] adoptive transfer of T-cells[11] and therapeutic tumour vaccines.[1]

In an attempt to expressly induce tumour-specific T-cell responses, a cryopreserved vaccine targeting the dominant immune epitopes of HER2 was developed (ADXS31-164/ADXS-HER2, Advaxis, Inc., Princeton, NJ). This therapeutic vaccine was generated using a highly attenuated, recombinant *Listeria monocytogenes* platform that also expressed a chimeric human HER2$^+$ fusion protein. Although this vaccine was developed for human use, escalating doses were safely administered to 18 dogs with naturally occurring HER2$^+$ OSA. Those that received standard-of-care amputation (or limb-spare) and platinum chemotherapy, followed by the vaccine, had a significantly longer survival time (956 days) compared to a historical control group of patients that received standard-of-care alone (423 days).[12]

In 2017, a lyophilized formulation of the live *Listeria* vector vaccine (the canine osteosarcoma vaccine, live *Listeria* vector [COV-LLV] (Elanco, formerly Aratana Therapeutics, Inc, Greenfield, IN)) was granted conditional licensure from the United States Department of Agriculture (USDA), Center for Veterinary Biologics.[13] An initial field safety study was completed in 33 dogs with non-metastatic OSA. COV-LLV was administered following amputation and chemotherapy. Transient, mild-to-moderate fever, decreased appetite, nausea, vomiting and blood pressure alterations were reported in most dogs. Occasionally, diarrhoea and lethargy were reported to persist for several days following administration of COV-LLV. Two severe adverse events (AEs) were reported.[14] Extended safety field studies of COV-LLV commenced, although these data have not yet been published. However, one case report published in 2019 details a patient who developed a *Listeria* abscess following the use of this therapeutic vaccine, implying significant safety concerns.[15]

The purpose of this study was to evaluate the safety of COV-LLV in a population of dogs with OSA who did not qualify for, or who were not enrolled in, the extended field safety study, compared to the AEs reported in the initial field safety study submitted to the USDA for conditional licensure.[14]

2 | METHODS

This observational study was completed by collecting case information from dogs receiving COV-LLV at sites participating in the safety trial, but not enrolled in that study. Clinicians willing to participate were asked to complete an electronic data capture record for each patient (REDCap 8.1.10, Vanderbilt University, Nashville, TN, 2020). Data collected included patient age, breed, gender, neuter status, weight, diagnosis, presenting clinical signs, staging information (blood work completed; chest radiographs, computed tomography and abdominal ultrasound findings; if metastasis was present), concurrent medications (including chemotherapy and other immunological therapies), concurrent treatments (including radiation therapy), why the vaccine was used (for primary treatment of a gross OSA, metastatic disease, and so on), number of vaccines administered, AEs noted during and after vaccine administration, outcome (if known) and comorbidities. Adverse events recorded included nausea, vomiting, diarrhoea, hyporexia, lethargy, hypersensitivity reactions, systolic hypertension (defined as >180 mmHg), systolic hypotension (defined as <80 mmHg), fever (defined as >39.5°C) and other events not defined by these categories. All AEs were graded using the Veterinary Cooperative Oncology Group - common terminology criteria for adverse events (VCOG-CTCAE) following chemotherapy in biological antineoplastic therapy in dogs and cats v1.1.[16] Data were collected and descriptive statistical analysis was completed.

2.1 | Cell line validation statement

No cell line was used in this study.

3 | RESULTS

Data were collected on 50 individual patients from 11 participating sites. Forty-nine (49) patients were included in analysis; one patient was excluded as a result of an incomplete REDCap record. Median age of the population was 8 years (range 3-15 years) with a median weight of 32.3 kg (range 16.2-94.5 kg). Thirty-eight (78%) patients were diagnosed with an appendicular OSA; 11 (22%) were diagnosed with an axial OSA (Additional population information available in Supplementary Table 1).

Initial staging of patients prior to vaccine use was under the purview of the attending clinician and varied, but typically included a complete blood count, chemistry panel and chest radiographs/computed tomography. Five (10%) of 48 patients with initial staging had evidence of metastatic disease prior to starting therapy for OSA (one patient was not fully evaluated). Three had evidence of pulmonary metastatic disease while two had lymph node involvement. Twelve

(25%) additional dogs developed metastatic disease after initial staging and prior to starting the OSA vaccine. Eleven of these dogs had pulmonary involvement while one had lymph node involvement.

3.1 | Previous treatment

Six patients (12%) had no treatment prior to receiving the COV-LLV. The remaining patients had a variety of treatments including intravenous (IV) chemotherapy (30), amputation (28), metronomic chemotherapy (8), bisphosphonates (6), stereotactic radiation therapy (5), incomplete surgical excision (3) and limb-salvage surgery (1). No prior immunotherapeutic use was reported. The most common IV chemotherapy administered was carboplatin. Additional chemotherapy included IV doxorubicin, metronomic satraplatin, metronomic cyclophosphamide, metronomic lomustine, toceranib phosphate and trametinib.

3.2 | Vaccine use

As reported by the participating clinicians, the COV-LLV was used following standard-of-care amputation and chemotherapy (22; 21 received carboplatin, 1 received alternating carboplatin and doxorubicin), for gross primary disease (11), for gross metastatic disease (5), for both gross primary and metastatic disease (5), concurrently with standard-of-care amputation and chemotherapy (4) or following amputation alone (2).

All patients received pre-vaccine medications as recommended for dogs in the field safety study,[14] including 2 hours of IV fluids at approximately 3 to 5 mL/kg/hr, maropitant (1 mg/kg IV or subcutaneously) and diphenhydramine (approximately 2 mg/kg intramuscularly). Carprofen (approximately 2.2 mg/kg subcutaneously) was used in 43/49 (88%) of patients. Two patients were already on an alternative non-steroidal, while the remaining four patients did not receive a non-steroidal for unknown reasons. Additional supportive medications included ondansetron (0.2 mg/kg IV) in 26 (53%) patients. One additional patient received oral trazodone. Three doses of the vaccine 3 weeks apart were planned for all patients. No patient received a booster vaccine or more than three vaccinations.

Thirty-three (67%) of dogs received the vaccine alone regardless of vaccine intent. Seven dogs (14%) received concurrent metronomic chemotherapy, 3 (6%) concurrent IV chemotherapy, 3 (6%) concurrent zoledronate, and one each received concurrent palliative radiation therapy, IV and metronomic chemotherapy, and both metronomic chemotherapy and zoledronate. These additional treatments were given because of progressive disease, pain management, owner wishes, other reasons not specified or a combination of these.

After the three doses of the OSA vaccine, 34 dogs (69%) received no additional therapy, 12 dogs (24%) received metronomic chemotherapy, 1 dog received additional IV chemotherapy and metronomic chemotherapy, 1 dog continued to receive zoledronate infusions and 1 dog received an amputation following pathologic fracture. These treatments were given because of progressive disease, pain management, owner wishes, other reasons not specified or a combination of these.

3.3 | AEs and safety

A total of 123 treatments were given to the 49 dogs included in this study (not all dogs completed all three planned vaccinations). During administration of the vaccine, there were 74 individual AEs noted; 60% of vaccine administrations were associated with an AE. These events included nausea, vomiting, diarrhoea, hyporexia, lethargy, hypertension and fever. No hypersensitivity reactions or episodes of hypotension were noted. After vaccination, a total of 72 individual AEs were noted; 59% of vaccine administrations were followed by AEs (Table 1).

The most common AEs during vaccination infusion were nausea, followed by lethargy and fever (Table 1). The majority of these AEs were considered mild (VCOG-CTCAE grade 1) and were self-limiting or resolved with supportive care (Table 2). Additional AEs noted during the vaccine infusion included VCOG-CTCAE grade 1: increased pain/limping at the primary tumour site (1), tachycardia (1) and tachypnea (1).

The most common AEs after vaccine administration were lethargy, nausea and fever (Table 1). The majority of these AEs were considered mild (VCOG-CTCAE grade 1) (Table 2). Additional AEs noted after vaccine infusion included VCOG-CTCAE grade 1: regurgitation, acute pain at the stifle in a dog with primary rib OSA (aspirates of the stifle were consistent with a neutrophilic inflammation that was culture negative), multiple subcutaneous nodules that progressed during vaccine use (cytology and/or histopathology were not pursued), neutropenia, polydipsia, abdominal discomfort, a self-limiting dry cough, a moist cough and general discomfort; VCOG-CTCAE grade 2: prolonged hyporexia, uncontrollable bleeding of an oral OSA, self-limiting ataxia and progressive neck pain; VCOG-CTCAE grade 4: paraparesis and non-weight bearing lameness. Although all of these clinical findings developed after vaccine infusion, it is not known if they were directly related to COV-LLV administration.

Of note, four patients (8%) developed *Listeria*-positive infections or had tissues that cultured positive for *Listeria*. The first patient was a 13-year-old spayed female Rottweiler mix who developed an abscess of the OSA amputation site (VCOG-CTCAE grade 3). Twelve days following the first infusion of COV-LLV, the patient started licking the previous amputation site and discharge was noted by the owner. The patient was presented for emergency care, where the abscess was cultured and flushed. The patient was treated successfully with pain medications and amoxicillin/clavulanic acid; it was elected to stop additional vaccinations due to this AE. The second patient was an 8.5-year-old spayed female Great Dane that developed left stifle effusion and pain following the first dose of the OSA vaccine (VCOG-CTCAE grade 3). Culture of the joint effusion was positive for *Listeria*. This patient was successfully treated with cephalexin and amoxicillin/clavulanic acid. The patient's owners and the attending clinician

TABLE 1 Adverse events during and after administration of the canine OSA vaccine, live *Listeria* vector

Treatment number	Total no of patients experiencing AEs	Nausea	Vomiting	Diarrhoea	Hyporexia	Lethargy	Hypertension	Fever	Other
AEs during administration									
1 (n = 49)	30	18	2	2	4	14	5	10	1
2 (n = 40)	23	15	3	1	2	14	4	11	1
3 (n = 34)	21	11	0	1	2	12	3	7	1
Total (n = 123)	74	44	5	4	8	40	12	28	3
AEs after administration									
1 (n = 49)	40	11	2	9	6	15	4	11	11
2 (n = 40)	22	7	1	2	3	5	0	8	4
3 (n = 34)	10	3	0	0	1	4	0	2	4
Total (n = 123)	72	21	3	11	10	24	4	21	19

Abbreviations: AEs, adverse events; OSA, osteosarcoma.

TABLE 2 Grade of adverse events noted during and after administration of the canine osteosarcoma vaccine, live *Listeria* vector

VCOG-CTCAE grade	Nausea	Vomiting	Diarrhoea	Hyporexia	Lethargy	Hypertension	Fever	Other
AEs during administration								
1	42	5	3	2	40	12	16	3
2	1			3			10	
3	1						2	
4								
5								
AEs after administration								
1	18	2	8	9	19	4	18	9
2	3	1	3		4		2	5
3				1	1		1	2
4								2
5								1

Abbreviations: AEs, adverse events; VCOG-CTCAE, Veterinary Cooperative Oncology Group-common terminology criteria for adverse events.

elected to continue with subsequent vaccinations despite this AE, and additional severe AEs were not noted for this patient. The third patient was a 12-year-old spayed female mixed breed dog that developed clinical signs consistent with a urinary tract infection 31 days following the third OSA vaccine (VCOG-CTCAE grade 2). A urine culture was completed and was positive for *Listeria*. This was successfully treated with amoxicillin. The fourth patient was a 6-year-old castrated male Bernese Mountain Dog that died unexpectedly one day following the third vaccine administration (VCOG-CTCAE grade 5). Necropsy demonstrated marked, multifocal, pulmonary and pleural fibrosis and moderate focal to diffuse alveolar histiocytosis with mild haemorrhage of both caudal lung lobes, with multifocal osteosarcoma emboli noted in the right caudal lung lobe. The lung lobes cultured positive for *Listeria*. These patients experienced other AEs, most commonly fever, during COV-LLV administration, that were considered mild (VCOG-CTCAE grade 1 or 2). Although these patients did not have genomic testing to confirm the *Listeria* present was the same strain as the COV-LLV *Listeria*, lack of other historical exposure to *Listeria* renders the COV-LLV the most likely source of infection.

The vaccine protocol was prematurely discontinued in 15 patients (30%). In nine patients (18%), the vaccine administration protocol was discontinued following the first vaccine administration. One patient developed increased neck pain, one developed paraparesis and one discontinued treatment as a result of a previously mentioned abscess formation. Four patients were euthanized due to disease progression. One of these patients was a previously mentioned patient with uncontrollable bleeding from an oral OSA; whether this bleeding was because of disease progression or secondary to vaccine use is unclear. One patient was lost to follow-up and in the final patient treatment was discontinued for an unspecified reason.

In six patients (12%) the vaccine administration protocol was discontinued following the second vaccination. One patient developed progressive cutaneous nodules of unknown aetiology (cytology and/or histopathology were not pursued); whether this was due to disease progression or the vaccine was not elucidated. Five patients

experienced progressive disease after the second vaccination; in four of these it was elected to discontinue treatment and in the final dog euthanasia was elected because of declining quality of life.

As a result of significant variation in COV-LLV treatment intent, follow-up evaluation, concurrent therapy and therapy used after vaccination, efficacy and impact of COV-LLV on survival time could not be assessed in this population of patients.

4 | DISCUSSION

The study reported herein aimed to evaluate and report on AEs observed during and after administration of the novel COV-LLV. During administration, AEs were mild, mostly consisting of VCOG-CTCAE grade 1 nausea, lethargy and fever. However, following administration several VCOG-CTCAE grade 3 AEs were observed. In addition, four dogs (8%) cultured positive for *Listeria* following administration of COV-LLV (VCOG-CTCAE other immunologic event grades 2, 3 and 5). This is the most concerning finding of this study, as the vaccine is significantly attenuated and should not cause clinically relevant infection.[12,17]

OSA is a devastating and aggressive disease in humans and dogs[1] and alternative treatment options are necessary to achieve more successful long-term outcomes. Harnessing the immune system is an attractive, plausible way to potentially increase survival times in those diagnosed with OSA.[1] Immunologic therapeutic approaches include the development of vaccinations to either non-specifically activate the hosts' innate immunity, or specifically stimulate the hosts' immune system.[18,19] Direct stimulation is frequently achieved using a bacterial vector, such as *Lactococcus*, *Salmonella*, *Shigella* or *Listeria*.[20]

Listeria is a gram-positive bacterium that typically infects epithelial cells of the small intestine. The body forms both a strong innate response to the intracellular *Listeria* via $CD4^+$ T-lymphocytes, and a strong adaptive response to future infections by activating $CD8^+$ T-lymphocytes.[19] Highly attenuated *Listeria*, achieved through deletion of one or more virulence genes and fused to a clinically relevant tumour-associated antigen, can be used to establish a therapeutic tumour vaccine for multiple tumour types. *Listeria*-based vaccines have been developed for the treatment of human cervical cancer, melanoma, breast cancer, prostate cancer and hepatocellular carcinoma.[19]

Mimicking what has been accomplished in human medicine, escalating doses of a human, attenuated, cryopreserved *Listeria* vaccine targeting HER2 (ADXS31-164/ADXS-HER2) were given to dogs with naturally occurring OSA following standard-of-care treatment.[12] In the initial clinical study of 18 patients without pre-existing metastatic disease, median survival time of those patients receiving the vaccine in addition to standard-of-care was significantly longer than historical controls. AEs reported in that group of patients were all transient and low grade, including fever, vomiting, arrhythmias, tachycardia, hypotension, hypertension, thrombocytopenia and neutrophilia within 24 hours of vaccine administration, and mild elevations in liver alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase. Blood cultures were performed on a subset of patients 24 hours following vaccine infusion, and all were negative.[12] All dogs in that study were pre-treated with an antiemetic (ondansetron) and diphenhydramine,[12] similar to the pre-treatment regimen in the initial field safety study[14] and the current study. The AEs reported in the initial field safety study were also transient, low-grade, and most commonly included fever, gastrointestinal signs and blood pressure alterations.[14]

These temporary, low-grade AEs are similar to what was found during the current study. Complete blood counts and chemistry profiles were not completed immediately after COV-LLV infusion, and thus evaluation of the previously reported immediate, transient neutrophilia and thrombocytopenia could not be completed. However, blood work on the day of each vaccination did not reveal significant cell line changes. Similarly, monitoring chemistry panels were not required during administration of the COV-LLV. For those patients who had chemistry panels available for review, significant changes attributable to the vaccine were not present.

A significant difference between the previous studies and the current study was the development of *Listeria*-positive cultures in 8% of the study population. These patients are in addition to the previously reported case that developed an intrathoracic *Listeria* abscess at a metastatic site following administration of the COV-LLV.[15]

Although listeriosis is an infrequently reported AE following therapeutic vaccination, three human cases have been documented following the use of a live *Listeria* therapeutic vaccine.[21-23] The *Listeria* used in the COV-LLV is highly attenuated by deletion of the virulence factor ActA, preventing the cell-to-cell spread.[17] This interrupts the intracellular lifecycle, but it does not prevent *Listeria* from being phagocytosed by antigen presenting cells, or intracellular replication. In addition, this deletion does not reduce the *Listeria's* ability to repair DNA damage or to replicate. It is possible that the *Listeria* in these cases was able to spread throughout the body via alternative virulence factors; the exact mechanism by which this might have occurred is unknown. Of note, one patient in the current study (that with the *Listeria*-positive pulmonary parenchyma on necropsy) and the previously reported thoracic abscess case[15] both developed *Listeria*-positive cultures at the site of metastatic OSA.[15] The role of metastatic OSA in the successful development of these *Listeria*-positive sites is unknown; it is possible that altered immune surveillance at the metastatic sites will have aided the growth of the *Listeria* by offering a protected niche for replication.[24]

Three dogs reported within this study developed *Listeria*-positive infections. A fourth patient was discovered to have *Listeria*-positive pulmonary parenchyma on necropsy. The clinically relevant infections developed up to 31 days post COV-LLV infusion, indicating that *Listeria*-based infections could develop at a significant time interval following administration, which is potentially one reason why no infections were reported in the previous study, as blood cultures were completed within 24 hours of vaccine administration.[12] The human cases were reported within hours, [22] days[21] and months[23] following administration, implying that *Listeria* infections could occur at any time following vaccine infusion.

It is possible that additional dogs in this population could have developed *Listeria* infections that were clinical and not investigated/cultured, subclinical or prevented by concomitant use of antibiotics. One dog was administered amoxicillin/clavulanic acid for unknown reasons. This is an acceptable treatment for listeriosis and thus could have masked or prevented an infection. An additional five dogs were receiving other antibiotics (one dog received cefpodoxime, four metronidazole). *Listeria* has been shown to be resistant to all cephalosporins,[25] so in this particular dog a masked infection is unlikely. The impact of metronidazole use is unknown. In humans, it is often included in an empiric cocktail of antibiotics for sepsis with an unknown focus, including listeriosis, but not as a specific recommendation for *Listeria* treatment.[26]

The formulation of the COV-LLV described in this study is a lyophilized form of the cryopreserved *Listeria* vaccine. Lyophilization facilitates storage at multiple types of veterinary facilitates. Although this technique is widely used to effectively distribute vaccines to the developing world where consistent cold storage may be impossible,[27] the impact of lyophilization on safety and efficacy of *Listeria*-based therapeutic vaccines is unknown. An expanded trial evaluating the anti-metastatic effects of the original, cryopreserved *Listeria* vaccine formulation previously reported[12,17] recently concluded recruitment.[28] Thus, at this time it is unclear if the infection rates between the two formulations are similar, or if lyophilization may potentially influence the safety of the vaccine.

The patients in this population were not standardized to treatment intent, concomitant treatments or staging, rendering evaluation of the impact of the COV-LLV on progression free interval or survival time impossible. In addition, collection of blood work and other diagnostics were not required, so additional AEs concerning blood dyscrasias or clinically silent infections could have been missed. Despite these limitations, the current study revealed that during COV-LLV infusions, AEs are typically mild and low-grade. However, there is a chance for the development of a clinically relevant *Listeria* infection, a severe AE that could potentially be life threatening. This finding also highlights the necessary precautions that need to be taken by the veterinarians and families of patients being treated with live vector vaccines to limit the potential zoonotic spread of disease. Since the collection of this safety data, the COV-LLV product licence has been terminated by the company following an assessment of the risks and benefits provided by the product (personal communication via CMJ with Elanco, January 2020).

ACKNOWLEDGEMENTS

The authors would like to acknowledge the clinicians and technical support staff at the following practices for their contribution of cases to this study: BluePearl Veterinary Partners–Waltham, Bridge Animal Referral Center, Cornell Vet Specialists, Hope Veterinary Specialists, Las Vegas Veterinary Specialty Center, Southwest Veterinary Oncology, VCA Katonah-Bedford Veterinary Center, Veterinary Cancer and Surgery Specialists, Veterinary Specialty Hospital, WestVet ER and Specialty.

CONFLICT OF INTEREST

C. M. J. is a paid consultant, speaker and advisory board member for Elanco. C. A. C. and P. J. B. are advisory board members for Elanco. M. L. M., C. D. T., C. A. C., P. J. B. and C. M. J. received clinical trial funding from Elanco (formerly Aratana Therapeutics, Inc.).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Margaret L. Musser https://orcid.org/0000-0003-4350-0614

REFERENCES

1. Wycislo KL, Fan TM. The immunotherapy of canine osteosarcoma: a historical and systematic review. *J Vet Intern Med*. 2015;29(3):759-769.
2. Vail D, Thamm D, Liptak J, eds. *Withrow and MacEwen's Small Animal Clinical Oncology*. 6th ed. St. Louis, MO: Saunders; 2019.
3. Hoption Cann SA, van Netten JP, van Netten C. Dr William Coley and tumour regression: a place in history or in the future. *Postgrad Med J*. 2003;79(938):672-680.
4. Lascelles BD, Dernell WS, Correa MT, et al. Improved survival associated with postoperative wound infection in dogs treated with limb-salvage surgery for osteosarcoma. *Ann Surg Oncol*. 2005;12(12):1073-1083.
5. Chen YU, Xu SF, Xu M, Yu XC. Postoperative infection and survival in osteosarcoma patients: reconsideration of immunotherapy for osteosarcoma. *Mol Clin Oncol*. 2015;3(3):495-500.
6. Liptak JM, Dernell WS, Ehrhart N, Lafferty MH, Monteith GJ, Withrow SJ. Cortical allograft and endoprosthesis for limb-sparing surgery in dogs with distal radial osteosarcoma: a prospective clinical comparison of two different limb-sparing techniques. *Vet Surg*. 2006;35(6):518-533.
7. Owen LN, Bostock DE. Effects of intravenous BCG in normal dogs and in dogs with spontaneous osteosarcoma. *Eur J Cancer*. 1974;10(12):775-780.
8. MacEwen EG, Kurzman ID, Rosenthal RC, et al. Therapy for osteosarcoma in dogs with intravenous injection of liposome-encapsulated muramyl tripeptide. *J Natl Cancer Inst*. 1989;81(12):935-938.
9. Dow S, Elmslie R, Kurzman I, MacEwen G, Pericle F, Liggitt D. Phase I study of liposome-DNA complexes encoding the interleukin-2 gene in dogs with osteosarcoma lung metastases. *Hum Gene Ther*. 2005;16(8):937-946.
10. Modiano JF, Bellgrau D, Cutter GR, et al. Inflammation, apoptosis, and necrosis induced by neoadjuvant fas ligand gene therapy improves survival of dogs with spontaneous bone cancer. *Mol Ther*. 2012;20(12):2234-2243.
11. Visonneau S, Cesano A, Jeglum KA, Santoli D. Adjuvant treatment of canine osteosarcoma with the human cytotoxic T-cell line TALL-104. *Clin Cancer Res*. 1999;5(7):1868-1875.
12. Mason NJ, Gnanandarajah JS, Engiles JB, et al. Immunotherapy with a HER2-targeting listeria induces HER2-specific immunity and demonstrates potential therapeutic Eefects in a phase I trial in canine osteosarcoma. *Clin Cancer Res*. 2016;22(17):4380-4390.
13. Aratana Therapeutics Granted Conditional License for a Canine Osteosarcoma Therapeutic [press release]. Leawood, KS: Aratana Therapeutics, Inc. 2017.
14. Aratana Therapeutics. Canine osteosarcoma vaccine, live Listeria vector package In. Leawood, KS. 2017.

15. Musser ML, Berger EP, Parsons C, Kathariou S, Johannes CM. Vaccine strain *Listeria monocytogenes* abscess in a dog: a case report. *BMC Vet Res*. 2019;15(1):467.
16. Veterinary cooperative oncology group - common terminology criteria for adverse events (VCOG-CTCAE) following chemotherapy or biological antineoplastic therapy in dogs and cats v1.1. *Vet Comp Oncol*. 2016;14(4):417-446.
17. Shahabi V, Seavey MM, Maciag PC, Rivera S, Wallecha A. Development of a live and highly attenuated *Listeria monocytogenes*-based vaccine for the treatment of Her2/neu-overexpressing cancers in human. *Cancer Gene Ther*. 2011;18(1):53-62.
18. Mougel A, Terme M, Tanchot C. Therapeutic cancer vaccine and combinations with antiangiogenic therapies and immune checkpoint blockade. *Front Immunol*. 2019;10:467.
19. Wood LM, Paterson Y. Attenuated *Listeria monocytogenes*: a powerful and versatile vector for the future of tumor immunotherapy. *Front Cell Infect Microbiol*. 2014;4:51.
20. Hollingsworth RE, Jansen K. Turning the corner on therapeutic cancer vaccines. *NPJ Vaccines*. 2019;4:7.
21. Denham JD, Lee DH, Castro M, et al. Two cases of disseminated infection following live organism anti-cancer vaccine administration in cancer patients. *Int J Infect Dis*. 2018;72:1-2.
22. Sacco JJ, Evans M, Harrington KJ, et al. Systemic listeriosis following vaccination with the attenuated *Listeria monocytogenes* therapeutic vaccine, ADXS11-001. *Hum Vaccin Immunother*. 2016;12(4):1085-1086.
23. Fares E, McCloskey CB, Gutierrez A, Princiotta M, Salinas LJ, Drevets DA. Vaccine strain *Listeria monocytogenes* bacteremia occurring 31 months after immunization. *Infection*. 2019;47(3):489-492.
24. Lussier DM, O'Neill L, Nieves LM, et al. Enhanced T-cell immunity to osteosarcoma through antibody blockade of PD-1/PD-L1 interactions. *J Immunother*. 2015;38(3):96-106.
25. Troxler R, von Graevenitz A, Funke G, Wiedemann B, Stock I. Natural antibiotic susceptibility of listeria species: *L. grayi*, *L. innocua*, L. ivanovii, *L. monocytogenes*, L. seeligeri and L. welshimeri strains. *Clin Microbiol Infect*. 2000;6(10):525-535.
26. Thonnings S, Knudsen JD, Schonheyder HC, et al. Antibiotic treatment and mortality in patients with *Listeria monocytogenes* meningitis or bacteraemia. *Clin Microbiol Infect*. 2016;22(8):725-730.
27. Adams GD. Lyophilization of vaccines: current trends. *Methods Mol Med*. 2003;87:223-244.
28. National Cancer Institute. Comparative Oncology Trials Consortium https://ccr.cancer.gov/comparative-oncology-program/trials. Accessed June 3, 2020.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Musser ML, Berger EP, Tripp CD, Clifford CA, Bergman PJ, Johannes CM. Safety evaluation of the canine osteosarcoma vaccine, live *Listeria* vector. *Vet Comp Oncol*. 2021;19:92–98. https://doi.org/10.1111/vco.12642