ANTICANCER RESEARCH *39*: 1699-1703 (2019)
doi:10.21873/anticanres.13275

# Safety Evaluation of Autologous Tissue Vaccine Cancer Immunotherapy in a Canine Model

RACHEL A. CROSSLEY[1*], ALYSSA MATZ[2*], TERRY DEW[3], ASHLEY KALINAUSKAS[3], NICOLE FAUCETTE[1], BRAD POFF[3], LAWRENCE K. SILBART[2] and MARK A. SUCKOW[3,4]

[1]*Department of Pathobiology and Veterinary Science, College of Agriculture, Health and Natural Resources, University of Connecticut, Storrs, CT, U.S.A.;*
[2]*Department of Molecular and Cell Biology, College of Liberal Arts and Sciences, University of Connecticut, Storrs, CT, U.S.A.;*
[3]*Torigen Pharmaceuticals, Inc., Farmington, CT, U.S.A.;*
[4]*Department of Biomedical Engineering, University of Kentucky, Lexington, KY, U.S.A.*

**Abstract.** *Background/Aim: Previous work in rodent models showed that an autologous tissue vaccine is both a safe and effective approach for treating cancer; however, as a translational step, safety must first be evaluated in a more clinically-relevant model. Materials and Methods: An autologous immunotherapy produced from resected tumors, was evaluated in a clinically-relevant canine model to assess safety. Ninety-three dogs with spontaneously occurring tumors received vaccination with inactivated autologous tumor tissue combined with an adjuvant of particulate porcine small intestinal submucosa extracellular matrix (SIS-ECM). Patients were followed to assess the occurrence of adverse events, overall survival, and tumor recurrence and/or metastasis. Results: A small number (12%) of patients experienced limited, mild pyrexia, injection site swelling, or lethargy, all resolving without clinical intervention.*
Conclusion: Autologous whole cell cancer immunotherapy can be used safely in the canine model of cancer and represents a safe approach for the treatment for cancer.

Improved understanding of the importance of immune regulation in cancer has led to increased work toward the development of immunotherapeutics for the treatment of cancer. Neoplastic tissue arises from genetic or epigenetic mutations that can manifest as proteins on the cell surface and which are referred to as tumor-associated antigens (TAAs). The immune system targets and destroys cells bearing these abnormal antigens as "foreign material", however, neoplastic cells can concurrently express regulator proteins that counteract the immunogenic properties of their TAAs. Such tissue creates a highly tolerogenic microenvironment, preventing effector immune cells from recognizing and/or killing their targets, allowing for completely unregulated proliferation and the progression of cancer. Immunotherapeutics heighten immune stimulation towards tumor antigens, bypassing suppression and activating cancer-killing cells systemically against primary and metastatic malignancies (1). Cancer immunotherapy has rapidly evolved as a viable option in human medicine, as both a primary and adjunctive treatment to classical approaches such as chemo- and radiation therapies.

Autologous tissue vaccines are a type of cancer immunotherapy that consist of cells harvested directly from a patient's own tumor, thus representing the full heterogeneity of a patient's unique tumor-associated antigen (TAA) profile. Tissue is mechanically dissociated, thus avoiding potential destruction of TAAs that might occur following enzymatic digestion of tissue. Following chemical inactivation of the tumor cells, the material is then combined with an immune adjuvant. With such an approach, cells do not undergo culture *in vitro*, a step which has been demonstrated *via* microarray analysis to result in alterations to the antigenic profile of cells (2). Furthermore, autologous tissue vaccines offer a safe and cost-effective alternative to chemo- and radiation therapy with promising results demonstrated in rodent cancer models. Using a unique autologous cancer tissue vaccine, a significant decrease in tumor growth and metastasis in rodent models was demonstrated, including a reduction in the incidences of *de novo* prostate tumor development by 90%; reduction in primary tumor regrowth by 65%; decrease of 60% in pulmonary metastasis incidence; and an overall extended

*These Authors contributed equally to this study.

*Correspondence to:* Mark A. Suckow, Department of Biomedical Engineering, University of Kentucky, Lexington, 40506 KY, U.S.A. Tel: +1 8592571117, e-mail: msuckow@uky.edu

*Key Words:* Autologous vaccine, cancer immunotherapy, dog, model, safety.

survival time (3-5). In addition, pre-clinical studies demonstrated tissue vaccines to be a useful adjunct to external-beam irradiation, yielding an additional 50% reduction in tumor mass (2, 6). Based on the TH1 cytokine profile observed in splenocytes re-stimulated *in vitro* with tumor cell lysates, the mechanism of action appears to be consistent with other immunotherapies to stimulate immunity to TAAs *via* the induction of cytotoxic T cells against foreign material (5). In studies using inbred rodents with induced cancer, use of autologous tissue vaccines has not been associated with any adverse outcomes; however, safety in a model that recapitulates more closely clinical disease associated with cancer in man has not yet been explored and is a logical step in the translational process.

Every year, around 4 million dogs are diagnosed with cancer, and nearly 50% of veterinarians report seeing cancer more frequently in their clinical caseload (7). A 2011 study found neoplastic disease to be the most prevalent cause of death in adult dogs, with 45% of dogs that live to 10 years or older dying of cancer (8). Because of the many similarities in clinical presentation and progression, spontaneous cancer in dogs is recognized as a model for studies of cancers affecting humans (9-12).

The studies described here were undertaken to evaluate the safety, *via* occurrence of adverse events, associated with use of an autologous tissue vaccine following surgical tumor debulking in the clinically relevant canine model. Specifically, the safety was evaluated in 93 dogs presenting with a wide variety of tumor types at a single veterinary surgical referral practice.

## Materials and Methods

*Study design*. Canine patients presenting with solid tumors at a surgical referral practice (Azzore Veterinary Specialists, Russellville, AR, USA) were invited to enroll in a prospective study to determine the safety of the autologous cancer vaccine beginning in March, 2015 and concluding in April, 2018. Enrollment was made without consideration to patient age, breed, cancer type, or stage of cancer; however, prior treatment with chemotherapy or radiation were used as exclusion criteria. A total of 93 dogs were administered autologous tissue vaccine following surgical excision of the primary and/or metastatic tumors, with part of each tumor submitted for histopathological determination of the cancer type. The remaining tissue was submitted to a commercial laboratory (Torigen Pharmaceuticals, Inc., Farmington, CT, USA) for processing to produce the autologous cancer vaccine. All surgeries were conducted by a surgeon board-certified by the American College of Veterinary Surgery (ACVS) (T. Dew). The primary goal of the tumor resection surgery was to maintain normal regional physiologic function; and a secondary goal was to attain surgical margins grossly free of neoplasia. The vaccine was administered subcutaneously near the closest draining peripheral lymph node to the excision site, in three 1 ml doses given at weekly intervals. As described below, all patients were subsequently monitored for adverse events and progression of tumor-associated disease. Initial follow-up exams were performed by either the surgeon or by the referring veterinarian.

*Vaccine preparation*. After surgical excision, unfixed tumor tissue was shipped overnight to the processing laboratory in a sterile container, on wet ice. Upon arrival, approximately 5 g of tissue were trimmed and processed. The tumor tissue was mechanically dissociated into Dulbecco's' Modified Eagle Medium (DMEM) (Corning life Sciences, Tewksbury, MA, USA; catalog number 15-017) and filtered through a 70 μm cell strainer (VWR, Radnor, PA, USA) to obtain a uniform cell suspension. The cell suspension was centrifuged at 1,000 × g for 5 min, creating a pellet formed of densely packed tumor material. The pellet was separated from the supernatant and resuspended in 5 ml of 2.5% glutaraldehyde (Alfa Aesar, Thermo Fisher Scientific Ward Hill, MA, USA) to form glutaraldehyde-fixed tumor (GFT) tissue. Following centrifugation, the pellet was washed thrice with 7.4 pH sterile phosphate buffered saline (PBS) (Quality Biological Inc., Gaithersburg, MD, USA; catalog number 114-056-101). The final GFT suspension was combined with 1 μg of small intestinal submucosa extracellular matrix (SIS-ECM) vaccine adjuvant (Cook Biotech, Inc., West Lafayette, IN, USA) in particulate form, then transferred to a sterile vial and shipped overnight on wet ice to the referring veterinarian for subcutaneous administration to the patient.

*Safety*. Safety was evaluated by observing animals immediately after vaccine administration and over the ensuing 3 months post-treatment. The referring veterinarian monitored for adverse events for 30 min following each of the three injections. At patient discharge, pet owners were educated on possible reactions and instructed to report any observed abnormalities immediately upon their occurrence or at any point over the ensuing three months. Adverse events were categorized using standardized nomenclature and severity plus relatedness to the test article; and these responses were scored by the attending veterinarian using a standardized system for scoring adverse events in dogs following biological therapy (13).

## Results

As shown in Table I, a variety of solid tumors of both mesenchymal (sarcoma) and epithelial (carcinoma) origin were treated during this analysis. Specifically, over 30 different tumor types were analyzed.

Of the 93 dogs treated with the autologous tissue vaccine, only twelve adverse events occurred in eleven animals (Table II). All of the adverse events were grade 1, on a scale of grades 1-5, scored using a standardized system by the attending veterinarian (13). Grade 1 adverse events were defined as “asymptomatic or mild symptoms; clinical signs or diagnostic observations only; or intervention not indicated.” Per the scoring system, recorded adverse events included: mild tissue edema at the site of administration (8 adverse events); mild administration site irritation; and lethargy/fatigue. All adverse events resolved without treatment. No other adverse events attributable to vaccination were observed during the interval between vaccine administration and the subsequent three months.

Table I. *Patients with adverse reactions to autologous cancer vaccine and outcomes (n=11 patients, 12 reactions).*

| ID | Cancer type | Patient demographics | | | Number of doses | Adverse reaction | Grade of reaction* | Safety outcome |
|---|---|---|---|---|---|---|---|---|
| | | Gender | Age (yrs) | Weight (lbs) | | | | |
| 1 | Oral melanoma (amelanotic) | FS± | 9 | 35 | 3 | AdS** swelling | 1 | Resolved No Tx±± |
| 2 | Recurrence | FS | 9 | 35 | 3 | AdS swelling | 1 | Resolved No Tx |
| 3 | Adenocarcinoma (anal sac) | FS | 9 | 40.8 | 3 | Lethargy 2nd day | 1 | Resolved No Tx |
| 4 | Osteosarcoma (palate) | MN† | 4 | 72.9 | 3 | Lethargy 2nd day | 1 | Resolved No Tx |
| 5 | Transitional cell carcinoma | FS | 12 | 24.4 | 3 | Lethargy 2nd day, AdS swelling /Nodules | 1 | Resolved No Tx |
| 6 | Adenocarcinoma (anal sac) | FS | 10 | 29.4 | 3 | AdS nodule | 1 | Resolved No Tx |
| 7 | Mast cell (Grade III) | FS | 13 | 20 | 3 | AdS irritation | 1 | Resolved No Tx |
| 8 | Squamous cell carcinoma | FS | 8 | 69.6 | 1 | Lethargy 2nd day | 1 | Resolved No Tx |
| 9 | Squamous cell carcinoma | FS | 11 | 75 | 3 | AdS swelling | 1 | Resolved No Tx |
| 10 | Unknown | FS | 9 | 10.5 | 3 | AdS nodule | 1 | Resolved No Tx |
| 11 | Mast cell (Grade III) | MN | 15 | 34.4 | 3 | AdS swelling | 1 | Resolved No Tx |
| 12 | Oral melanoma | FS | 12 | 11.9 | 3 | AdS nodule | 1 | Resolved No Tx |

*Graded on a scale of 1-5, scored using a standardized system (VCOG-CTCAE) (1 being least severe, 5 being most severe) (19). ±FS: Female spayed; †MN: male neutered (intact animals have a higher risk of certain types of cancers). **AdS: administration site; ±±Tx: treatment.

## Discussion

Though the goal of surgical tumor excision is the ablation of neoplastic tissue, incomplete surgical margins are common and may result in tumor recurrence. For that reason, therapies that are adjunctive to surgery are of great interest. Current adjunctive methods include chemotherapy and radiation therapy; however, both have multiple potential adverse effects that can diminish the patient's quality of life (14-17). The purpose of the work described here is to extend the safety results of earlier studies in rodents using the translational canine cancer model. The "all-comers" enrollment approach used in this study yielded a wide variety of tumor types and histological grades. Our results demonstrated that an autologous tissue vaccine can be used safely when combined with surgical tumor reduction. In the present study, we demonstrated that treatment of 93 post-resection canine cancer patients with autologous tissue vaccine cancer immunotherapy resulted in only 12 adverse events involving 11 dogs. All adverse events were mild and resolved rapidly with no further treatment. This result supports the idea that the use of autologous whole cell preparations for treatment of a wide variety of cancers poses little risk to the patient. To create the autologous cancer immunotherapy, a patient's surgically excised tumor sample was mechanically separated into a suspension, then deactivated and cross-linked using glutaraldehyde treatment. This approach avoids antigen epitope alterations associated with *in vitro* culture of the harvested cells. Further, because enzymatic degradation is not used to dissociate the tissue, vaccine components include an antigenically rich repertoire of immune targets that involve not only neoplastic cells, but also the tumor stromal components which act as a lattice to facilitate tumor growth and progression (2).

Table II. *Various solid canine cancer types/stages (n=93).*

| Cancer types treated by locations | Number enrolled |
|---|---|
| Carcinoma | 36 |
| Anal tumors | 7 |
| Mammary | 2 |
| Liver | 5 |
| Nasal | 3 |
| Bladder | 8 |
| Oral | 4 |
| Thyroid | 3 |
| Other | 4 |
| Sarcoma | 35 |
| Spleen | 8 |
| Oral | 5 |
| Skin | 18 |
| Liver | 1 |
| Bone | 3 |
| Other | 22 |

Though the study described here was designed to evaluate safety rather than efficacy, previous efficacy data in rodent models suggest that the whole cell autologous cancer immunotherapy evaluated here works by stimulating

production of immunostimulatory cytokines characteristic of TH1 cell-mediated immunity (5, 18). By providing antigens unique to the patient's own tumor, it is reasonable to expect that the resulting immune response is primed to recognize those antigens that are most likely to be of clinical relevance to that tumor. In view of the strong safety profile demonstrated in the study described here, autologous whole cell cancer immunotherapy warrants further study and development. Future studies are needed to establish efficacy in a clinically relevant canine model as a step toward translation.

The vaccine evaluated for safety in this study included a proprietary adjuvant consisting of porcine SIS-ECM, a material that has been shown to promote leukocyte recruitment and direct phagocytosis by innate immune cells (19). Further, studies suggest that the SIS-ECM prolongs epitope retention by antigen presenting cells (APCs), which use TAAs to select for maturation of CD8+ cytotoxic T lymphocytes (CTLs) bearing the complementary T-cell receptors (18, 19). Clonal expansion and activation of these specific CTLs following treatment with autologous vaccines directly correlates to cancer regression (20).

The similarities between canine cancer and that of man are significant (10-12). For example, dogs generally share the same environment as humans and are exposed to the same environmental carcinogenic influences. Furthermore, many canine cancers are characterized by familial predisposition, similar to those of humans. This homology of cancer between dogs and humans suggests that the safety observed in canine patients in the study described here could likely translate to human patients. As a consequence, the data from this study suggest that use of whole cell autologous immunotherapy for cancer would pose no risk when used in treatment of cancer in man.

In summary, using the clinically relevant canine model, we demonstrated that an autologous whole-cell immunotherapy can be safely used across a spectrum of tumor types. Additional work will be needed to confirm in this model the efficacy results noted in preclinical rodent models.

## Authors' Contributions

R.A.M, A.M., and N.F. coordinated the collection and interpretation of the data; T.D performed surgical excision of tumors and provided animal care; A.K. B.P, and L.K.B. were responsible for processing of samples and developing methods for production of the immunotherapeutic; and M.A.S. evaluated the data and wrote the manuscript. All Authors critically reviewed the article.

## Acknowledgements

The Authors thank Alexus Maschinot, Lauren Klein and Meaghan Bolles for all their help with tumor processing; Isabella Raymond for assisting with data analysis; the Technological Incubation Program (TIP) at the University of Connecticut for providing wet and dry labs, offices and co-working spaces in Farmington, Connecticut as well as access to instrumentation, research services and collaboration with scientific experts; and Connecticut Innovations and UConn Innovation Fund for funding this project.

## References

1 Pandya P: The immune system in cancer pathogenesis: potential therapeutic approaches. J Immunol Res *12*: 273943, 2016. PMID: 28116316. DOI: 10.1155/2016/4273943

2 Suckow MA, Heinrich J and Rosen ED: Tissue vaccines for cancer. Expert Rev Vaccines *6*: 925-937, 2007. PMID: 18377356. DOI: 10.1586/14760584.6.6.925

3 Suckow MA, Wolter WR and Pollard M: Prevention of *de novo* prostate cancer by immunization with tumor-derived vaccines. Cancer Immunol Immunother *54*: 571-576, 2005. PMID: 15685450. DOI: 10.1007/s00262-004-0612-y

4 Suckow MA, Hall P, Wolter W Sailes V and Hiles MC: Use of an extracellular matrix material as a vaccine carrier and adjuvant. Anticancer Res *28*: 2529-2534, 2008. PMID: 19035274.

5 Suckow MA, Wolter WR and Sailes VT: Inhibition of prostate cancer metastasis by administration of a tissue vaccine. Clin Exp Metastasis *25*: 913-918, 2008. PMID: 18821060. DOI: 10.1007/s10585-008-9213-z

6 Suckow MA, Wolter WR and Sailes VT: Immunization with a tissue vaccine enhances the effect of irradiation on prostate tumors. In Vivo *22*: 171-177, 2008. PMID: 18468400.

7 Kidd C: The many challenges of veterinary oncology. Can Vet J *49*: 1132-1135, 2008. PMID: 19183739.

8 Fleming JM, Creevy KE and Promislow DEL: Mortality in North American dogs from 1984 to 2004: an investigation into age, size, and breed-related causes of death. J Vet Intern Med *25*: 187-198, 2011. PMID: 21352376. DOI: 10.1111/j.1939-1676.2011.0695.x

9 Fan TM and Stelting KA: Exploring the potential utility of pet dogs with cancer for studying radiation-induced immunogenic cell death strategies. Front Oncol *8*: 680, 2019. PMID: 30697532. DOI: 10.3389/fonc.2018.00680

10 Ranieri G, Gadeleta CD, Patruno R, Zizzo N, Daldone MG, Hansson MG, Paradiso A and Ribatti D: A model for study of human cancer: spontaneous occurring tumors in dogs. Biological features and translation for new anticancer therapies. Crit Rev Oncol Hematol *88*: 187-197, 2013. PMID: 23561333. DOI: 10.1016/j.critrevonc.2013.03.005

11 Paoloni M and Khanna C: Translation of new cancer treatments from pet dogs to humans. Nat Rev Cancer *8*: 147-156, 2008. PMID: 18202698. DOI: 10.1038/nrc2273

12 LeBlanc AK, Breen M. Choyke P., Dewhirst M, Fan TM, Gustafson DL, Helman LJ, Kastan MB, Knapp DW, Levin WJ, London C, Mason N, Mazcko C, Olson PN, Page R, Teicher BA, Thamm DH, Trent JM, Vail DM and Khanna C: Perspectives from man's best friend: National Academy of Medicine's Workshop on Comparative Oncology. Sci Transl Med *8*: 324ps5, 2016. PMID: 26843188. DOI: 10.1126/scitranslmed.aaf0746

13 Veterinary Cooperative Oncology Group: Veterinary Cooperative Group – a common terminology criteria for adverse events (VCOG-CTCAE) following chemotherapy or biological antineoplastic therapy in dogs and cats. Vet Comp Oncol *14*: 417-446, 2016. PMID: 28530307. DOI: 10.1111/vco.283

14 Adams VR: Adverse events associated with chemotherapy for common cancers. Pharmacotherapy *20*: 96S-103S, 2000. PMID: 10905684.
15 Chopra D, Rehan HS, Sharma V and Mishra R: Chemotherapy-induced adverse drug reactions in oncology patients: a prospective observational study. Indian J Med Paediatr Oncol *37*: 42-46, 2016. PMID: 27051157. DOI: 10.4103/0971-5851.177015
16 Yang M and Moon C: Neurotoxicity of cancer chemotherapy. Neural Regen Res *8*: 1606-1614, 2013. PMID: 25206457. DOI: 10.3969/j.issn.1673-5374.2013.17.009
17 Singh M, Alavi A, Wong R and Akita S: Radiodermatitis: a review of our current understanding. Am J Clin Dermatol *17*: 277-292, 2016. PMID: 27021652. DOI: 10.1007/s40257-016-0186-4
18 Chowdhury R, Aachoui Y and Ghosh SK: Effects of small intestinal submucosa (SIS) on the murine innate immune microenvironment induced by heat-killed Staphylococcus aureus. PloS One *7*: e48724, 2012. PMID: 23189134. DOI: 10.1371/journal.pone.0048724
19 Aaouchi Y and Ghosh SK: Immune enhancement by novel vaccine adjuvants in autoimmune-prone NZB/W F1 mice: relative efficacy and safety. BMC Immunol *12*: 61, 2011. PMID: 22024358. DOI: 10.1186/1471-2172-12-61
20 Ott PA, Hu Z, Keskin DB, Shukla SA, Sun J, Bozym DJ, Zhang W, Luoma A, Giobbie-Hurder A, Peter L, Chen C, Olive O, Carter TA, Li S, Lieb DJ, Eisenhaure T, Gjini E, Stevens J, Lane WJ, Javeri I, Nellaiappan K, Salazar AM, Daley H, Seaman M, Buchbinder EI, Yoon CH, Harden M, Lennon N, Gabriel S, Rodig SJ, Barouch DH, Aster JC, Getz G, Wucherpfennig K, Neuberg D, Ritz J, Lander ES, Fritsch EF, Hacohen N and Wu CJ: An immunogenic personal neoantigen vaccine for patients with melanoma. Nature *547*: 217-221, 2007. PMID: 28678778. DOI: 10.1038/nature22991

*Received February 20, 2019*
*Revised March 27, 2019*
*Accepted March 27, 2019*