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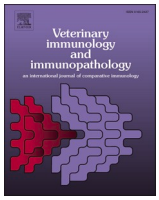

Design of a randomized, placebo-controlled study evaluating efficacy and safety of a cancer preventative vaccine in dogs

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ABSTRACT

Preventative anti-cancer vaccination strategies have long been hampered by the challenge of targeting the diverse array of potential tumor antigens, with successes to date limited to cancers with viral etiologies. Identification and vaccination against frameshift neoantigens conserved across multiple species and tumor histologies is a potential cancer preventative strategy currently being investigated. Companion dogs spontaneously develop cancers at a similar incidence to those in people and are a complementary comparative patient population for the development of novel anti-cancer therapeutics. In addition to an intact immune system with tumors that arise in an autochthonous tumor microenvironment, dogs also have a shorter lifespan and temporally compressed tumor natural history as compared to humans, which allows for more rapid evaluation of safety, immunogenicity, and efficacy of cancer vaccination strategies. Here we describe the study protocol for the Vaccination Against Canine Cancer Study (VACCS), the largest interventional cancer clinical trial conducted in companion dogs to date. In addition to safety and immunogenicity, the primary endpoint of VACCS is the cumulative incidence (CI) of dogs developing malignant neoplasia of any type at the end of the study period. Secondary endpoints include changes in incidence of specific tumor types, survival times following neoplasia diagnosis, and all-cause mortality.

1. Introduction

Cancer remains the second leading cause of death of people in the United States (Murphy et al., 2021). Despite this, cancer survivorship is increasing in the U.S. due to advances in screening, detection and treatment resulting in increasing costs of cancer care, which is projected

Abbreviations: CBC, complete blood count; CI, cumulative incidence; CSU, Colorado State University; DSMB, Data Safety and Monitoring Board; FS, frameshift; FSP, frameshift peptides; GMP, Good Manufacturing Practice; REDCap, Research Electronic Data Capture; PT, prothrombin time; PTT, partial thromboplastin time; UCD, University of California, Davis School of Veterinary Medicine; UW, University of Wisconsin – Madison School of Veterinary Medicine; VACCS, Vaccination Against Canine Cancer Study; VCOG-CTCAE, Veterinary Cooperative Oncology Group – Common Terminology Criteria for Adverse Events.

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to increase from $183 billion in 2015 to $246 billion by 2030 (Mariotto et al., 2020). Few efforts have been directed to cancer prevention beyond lifestyle changes such as smoking-cessation and decreasing sun exposure. Preventative cancer vaccines have not been widely investigated due to the largely personal DNA mutations in tumors. The successes of vaccines against human papilloma virus and hepatitis B virus highlight how preventative vaccination strategies are beneficial in reducing cancer development after infection with these viruses. Other anti-cancer vaccination strategies have primarily focused on developing therapeutic vaccines for specific tumor antigens; however, success has been limited to date (Saxena et al., 2021). Newer approaches have focused on targeting tumor neoantigens to develop personalized cancer therapeutic vaccines (Ott et al., 2017, Wang et al., 2022). These neoantigens are produced by a number of mechanisms including somatic mutations in DNA coding regions or frameshift mutations from microsatellite instability.

Our group has identified frameshift (FS) neoantigens that arise from errors in RNA processing, such as INDELs in microsatellites during transcription and mis-splicing of exons, some of which are conserved not only across tumor histologies, but also across species (human, dog, and mouse) (Shen et al., 2019). We have developed an array of predicted RNA error-derived frameshift peptides (FSPs) to identify antibody-bound FSPs in serum samples and efficiently detect these individual antibody responses (Zhang et al., 2018). Immunization with these neoantigens results in humoral and cell-mediated responses in purpose-bred laboratory dogs with no significant adverse effects noted (unpublished data). These data suggest the use of FS neoantigen vaccines may be a strategy for creating pan-cancer preventative vaccines that are agnostic of histologic type.

Companion (pet) animals spontaneously develop cancers that frequently have significant similarity to those that arise in people; therefore, companion dogs with cancer can be a complementary parallel patient population to assist in development of novel anti-cancer therapeutics and their inclusion is becoming more common (LeBlanc et al., 2016a, LeBlanc et al., 2016b, LeBlanc and Mazcko, 2020). As cancers in dogs arise spontaneously in the face of both an autochthonous tumor microenvironment and an intact immune environment, companion dogs are well-suited to evaluate novel immunotherapies, including vaccines, that are being developed for use in people (LeBlanc and Mazcko, 2020). Additionally, the shortened lifespan of companion dogs as well as the accelerated course of tumor progression as compared to humans allows conduct of interventional trials to occur on a condensed timeline. We propose that companion dogs can serve as an excellent parallel patient population to gain preliminary data regarding the safety, immunogenicity, and efficacy of a cancer preventive vaccine.

This paper outlines the methodology used for the Vaccination Against Canine Cancer Study (VACCS). The objective of the VACCS trial is to evaluate the efficacy and safety of a novel frameshift peptide-derived vaccine for the prevention of tumor development in dogs. The primary endpoint is the cumulative incidence (CI) of dogs developing malignant neoplasia of any type at the end of the study period. Secondary endpoints include evaluation of adverse effects, immune response, changes in incidence of specific tumor types, survival times following neoplasia diagnosis, and all-cause mortality.

2. Materials and methods

2.1. Study population

Healthy, client-owned companion dogs without a previous or current cancer diagnosis are recruited into this randomized, double-masked, placebo-controlled, prospective trial at three study sites – Colorado State University (CSU) Flint Animal Cancer Center, University of Wisconsin – Madison School of Veterinary Medicine (UW), and University of California, Davis School of Veterinary Medicine (UCD). We seek to enroll a canine patient population that is most likely to develop cancer-related events during the 5-year study time frame. To accomplish this, age is restricted to middle-aged to older dogs and inclusion limited to mixed breed dogs and breeds over-represented for death due to cancer (Dobson, 2013). Additionally, breeds at increased risk for specific tumor types but not overall increased risk for cancer are included (e.g., Scottish terriers and bladder cancer) (Knapp et al., 2014). See supplemental data (S1 Appendix) for full list of eligible breeds. The study is approved by and carried out in strict accordance with the Institutional Care and Use Committee (IACUC) and/or the Clinical Review Board at all three sites (CSU approval #585; UW approval #V-006039; UCD approval #20463).

To screen for general health and occult neoplasia, dogs are required to have a wellness examination performed by a veterinarian within 12 months prior to study entry and three years of medical records available for review by the study team to ensure there is no prior suspicion or diagnosis of malignant neoplasia by the primary care veterinarian. Dogs with a definitive history or strong suspicion of malignant neoplasia are excluded; however, dogs with a previous diagnosis of histologically benign neoplasms remain eligible for enrollment. Dogs with immunosuppressive conditions or receiving immunosuppressive therapy, or with concurrent co-morbidities with the potential to prevent 5 years of follow-up, are also excluded. At the initial study screening visit, a physical examination (by a study site veterinarian) is performed with measurement and fine-needle aspirate cytological assessment of all accessible dermal and subcutaneous masses. Complete blood count (CBC), chemistry profile, and prothrombin time (PT) and partial thromboplastin time (PTT) are performed to ensure adequate bone marrow and organ function. Additional cancer screening with three-view thoracic radiographs and abdominal ultrasound with imaging evaluated by a board-certified veterinary radiologist are also completed. Lesions noted with these imaging diagnostics where a neoplastic process is considered possible but cannot completely be ruled out are eligible for re-screening in 4–6 weeks to reassess these lesions; dogs whose lesions are unchanged, resolved, or evaluated cytologically as benign are then eligible for study enrollment. Full inclusion and exclusion criteria are outlined in Table 1.

Table 1
Vaccination Against Canine Cancer Study (VACCS) Inclusion and Exclusion Criteria.

INCLUSION CRITERIA
- Between the ages of 5.5- and 11.5 years old on Day 0
- Mixed breed dogs or eligible breeds (see Supplemental Data)
- Received a wellness exam from a veterinarian within 12 months of enrollment
- 3 years of medical records available for review
- Adequate organ function as determined by:
  - Absolute neutrophil count > 2000 cells/uL
  - Hematocrit > 35%
  - Platelet count > 125,000/ uL
  - Normal serum creatinine and bilirubin
  - Transaminases (ALT, AST) < 2x the ULN
- General Performance score of 0 on Day 0
- Signed owner informed consent

EXCLUSION CRITERIA
- A history of any previous malignant neoplasm
- Current evidence of likely, probable, or definite malignant neoplasia on pre-enrolment screening
- Concurrent co-morbidities which have the potential to interfere with the ability to follow a patient for 5 years
- Dogs undergoing treatment with immunosuppressive therapy
- Dogs previously diagnosed with hyperadrenocorticism
- Currently enrolled in another interventional clinical trial
- Owned by an Investigator or their staff
- Pregnant, lactating, or intended for breeding (male or female)
- General Performance score > 0 on Day 0

ALT, alanine transaminase; AST, aspartate transaminase; ULN, upper limit of normal.

2.2. Sample size determination, randomization and masking

The primary hypothesis of this study is that the study vaccine will be safe and reduce the CI of dogs developing malignant neoplasia by at least 30% as compared to the control arm. A total sample size of 800 eligible dogs is proposed for this trial as this sample size is adequate for detecting the anticipated decrease of at least 30% in the CI for the development of malignant neoplasia of any type in the vaccination arm when compared to the control arm at the two-sided 0.05 significance level. Table 2 shows the attainable power level for rejecting the null hypothesis that the rate ratio of the CI is one using a stratified log-rank test accounting for competing risks under the following assumptions: (1) a two-sided 0.05 significance level, (2) a sample size of 400 eligible dogs per study arm, (3) an attrition rate/unevaluable rate of 10%, (4) an anticipated CI for the development of malignant neoplasia of any type of 25% in the control arm at the end of the follow-up period, (5) 30–50% decrease in the CI for the development of malignant neoplasia of any type in the vaccination arm when compared to the control arm, (6) CIs for competing risks ranging between 50% and 60% in both the control and vaccination arm, (7) a total study period of 5 years including a 2 year accrual period, (8) uniform accrual pattern over 2 years, (9) time to diagnosis of malignant neoplasia and time to competing risks follow an exponential distribution, and (10) an interim analysis for superiority at the end of years 2, 3 and 4 using a Hwang-Shih-DeCani spending function (3 interim analyses plus 1 final analysis) with $\gamma = -4$. It is assumed that all dogs would have immunological responses to the vaccine based on results from purpose-bred laboratory dogs (unpublished data).

With the proposed sample size of 800 dogs, the anticipated decrease of at least 30% in the CI for the development of malignant neoplasia of any type in the vaccine treatment arm will be detected with 80–99% power if the CI for the competing risks is at least 55%. If the CI for the competing risks is between 50% and 55%, then the anticipated reduction of 30% in the CI for the development of malignant neoplasia of any type will be detected with at least 77% power at the two-sided 0.05 significance level. These calculations are based on the log-rank test accounting for competing risks, assuming the time to the diagnosis of malignant neoplasia at any time and time to competing risk failures are exponentially distributed.

2.3. Vaccine design and randomization

Frameshift neoantigens included in the vaccine were selected using two methods. First, candidates were identified by sequencing dog tumor RNA and identifying mis-spliced transcripts. These four encoded FSPs were shown to provide disease protection in several mouse tumor models (Shen et al., 2019, Zhang et al., 2018, Peterson et al., 2020). The remaining vaccine candidates were identified by screening dog cancer serum samples for specific antibody binding to microarrays displaying informatically predicted FSPs as previously described (Zhang et al., 2018). Those peptides showing the highest frequency of positive antibody signal across samples from dogs with the 8 most common cancers were identified. These candidates represent shared FS neoantigens. Bioinformatic filters were applied to eliminate candidates with high similarity to self-proteins and include those predicted to be highly immunogenic and to have a human homolog. To minimize the inclusion of possibly unannotated normal proteins, FS neoantigens longer than 100 amino acids were excluded, except for one candidate with one of the highest frequencies of antibody positivity.

Table 2
Attainable power level for the Vaccination Against Canine Cancer Study (VACCS). Attainable power level for detecting a decrease of at least 30% in the cumulative incidence (CI) for the development of malignant neoplasia of any type at the two-sided 0.05 significance level under various scenarios with a total sample size of 800 eligible dogs.

	Relative reduction in CI for the development of malignant neoplasia of any type in vaccination arm compared to control arm				
CI for competing risks	30%	35%	40%	45%	50%
50%	77%	88%	95%	98%	> 99%
55%	80%	91%	96%	99%	> 99%
60%	84%	93%	98%	99%	> 99%

The vaccine developed for this trial consists of two different formulations in a prime-boost regimen: plasmid DNA vaccine primes and peptide vaccine boost. The vaccine arm DNA vaccine encodes 31 FS neoantigens strung together on 2 nanoplasmids (Borggren et al., 2015), comprising a total of 1345 amino acids. The placebo arm contains a DNA plasmid encoding an irrelevant peptide (Chambers and Johnston, 2003). As a genetic adjuvant, a DNA plasmid encoding dog granulocyte-macrophage colony-stimulating factor (GM-CSF) is included in both vaccine and placebo. The vaccine arm peptide vaccine contains 20 of the 31 neoantigens as synthetic peptides ranging in length from 21 to 32 amino acids. The placebo arm peptide vaccine contains the irrelevant peptide encoded by the placebo arm DNA plasmid. Hiltonol is included as peptide adjuvant for both arms (Aso et al., 1985, Sultan et al., 2020).

Both the DNA and peptide vaccines are manufactured using non-Good Manufacturing Practice (GMP) material from GMP certified manufacturers. The DNA vaccine is produced and packaged by Aldevron (Fargo, ND), and peptides for the trial are synthesized by CPC Scientific (San Jose, CA). The peptide vaccine is produced and packaged in-house (Calviri, Inc, Phoenix, AZ) and the sterility and endotoxin-free character of both vaccines are tested by Pace Analytical (Minneapolis, MN). Hiltonol, a GMP product, is provided by Oncovir, Inc. (Washington, DC).

A randomization table is generated for each study site with two categories (active versus placebo vaccine) and dogs are randomized 1:1 to receive either the active or placebo vaccine. Masking of all study investigators and staff at the three study sites, pet owners, and statisticians is maintained for the duration of the study.

2.4. Study visits, sample collection and data capture

Fig. 1 outlines the study schema. Once eligibility is determined and randomization occurs, dogs are administered their initial vaccine series every 2 weeks for 4 vaccinations. The first two vaccines in the series are DNA vaccine or placebo (500 μg in 100 μL phosphate buffered saline (PBS)) administered intradermally via a PharmaJet Tropis® ID (Golden, CO, USA) injection device in the medial thigh. The 3rd and 4th vaccines in the booster series and all subsequent annual boosters are peptide vaccines (FSPs) or placebo (225 μg) in 500 μL PBS, administered i.m. using a PharmaJet Stratis® IM/SC (Golden, CO, USA) injection device. Hiltonol adjuvant (180 μg in 100 μL) is administered i.m. immediately prior to peptide vaccination in the same location as the peptide vaccine administration. Fur is clipped from all sites of vaccination prior to administration to ensure adequate contact of the device with the skin and to allow for monitoring of locoregional reaction and adverse events related to the vaccine.

Serum, plasma, and whole blood for peripheral blood mononuclear cell (PBMC) isolation are collected prior to the first vaccination, at the 4th vaccination (week 6), and at the 6-month study visit for immune response assessment. Dogs are evaluated every 6 months for a physical examination with routine CBC, biochemical profile, urinalysis, and PT/PTT performed annually. Serum and plasma are collected at every 6-month visit. Dog owners are encouraged to work with their primary care veterinarians between study visits for routine wellness care and medical issues that arise during the study and instructed to return to the study site if a neoplastic condition is suspected or diagnosed. Dogs that develop tumors during study participation have PBMC isolated and cell-free DNA collected at diagnosis when feasible. If tumor resection is performed at the study sites, flash frozen and formalin-fixed paraffin-embedded (FFPE) samples of normal and tumor tissue is collected, provided that the tumor is large enough to collect additional samples.

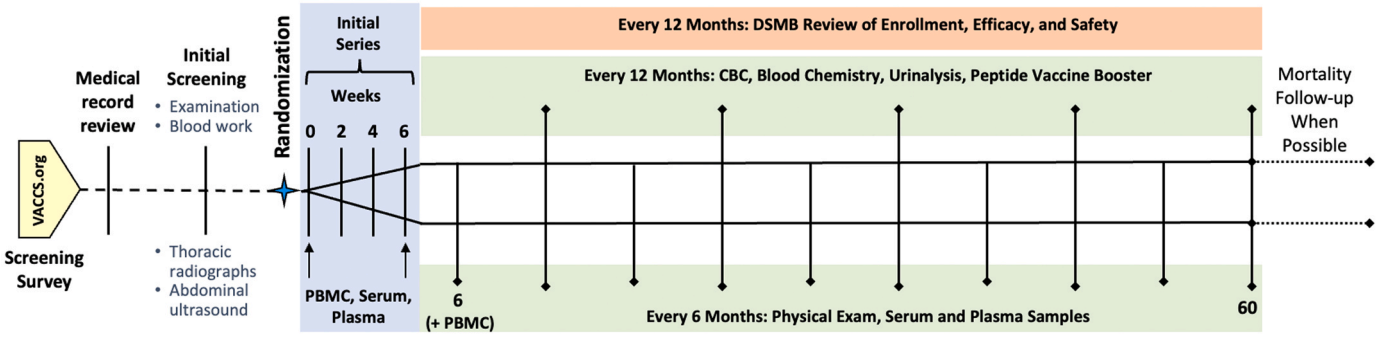

Fig. 1. Study Timeline. Dogs are screened for enrollment using a REDCap survey to ensure age, breed, and geographic location met eligibility criteria. Medical records from the previous three years are then obtained from the dog's primary care veterinarian and reviewed for previous cancer diagnoses or other medical history that may prevent participation throughout this 5-year prospective trial. Following medical record review, dogs are evaluated at one of the study sites for screening physical examination and diagnostics. If remaining eligibility criteria are met, dogs receive the initial vaccine booster series every two weeks followed by study visits every 6 months with vaccine booster administered annually.

Necropsy examination is requested at the time of death.

The PBMCs from the 6-month and the tumor detection timepoints are being utilized to monitor the VACCS-specific T cell responses to determine the cytokines (IFN-gamma and TNF-alpha) in CD4 + and CD8 + T cells in response to the VACCS peptide pool by flow cytometry. Peptide mapping is also being performed via interferon-gamma ELISpot assays. Serum and cell-free DNA are being collected for exploratory purposes. Sections from FFPE embedded tumors will be reviewed by a single pathologist at study completion. Although not included in the current protocol, post-hoc analysis of snap-frozen tumor tissue may include RNA sequencing of tumors that develop, with comparison of those developing in the vaccine arm versus those in the placebo arm for expression of vaccine-targeted neoantigens as well as other transcripts associated with immune avoidance.

Study data are collected and managed using REDCap electronic data capture tools hosted at the Colorado Clinical and Translational Sciences Institute (Harris et al., 2009). REDCap (Research Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies, providing: 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources. An electronic survey is automatically sent from REDCap to pet owners one week after each vaccination to assess for possible vaccine related adverse events. Adverse events are graded for severity using the Veterinary Cooperative Oncology Group – Common Terminology Criteria for Adverse Events (VCOG-CTCAE) v1.1 (doi: 10.1111/vco.283). As the VCOG-CTCAE was developed primarily to assess adverse events that may occur secondary to antineoplastic therapies, the FDA Guidance for Toxicity Grading for Preventative Vaccine Clinical Trials has been adapted to assess local reactions to vaccination (https://www.fda.gov/regulatory-information/search-fda-guidance-documents/toxicity-grading-scale-healthy-adult-and-adolescent-volunteers-enrolled-preventive-vaccine-clinical).

2.5. Study oversight

A Data Safety and Monitoring Board (DSMB) consisting of three members is convened annually to review study enrollment, patient demographics, and adverse event data. Additionally, masked interim efficacy analysis takes place in the 3rd and 4th years and is reviewed by the DSMB. Results from these analyses will be reported in the future.

3. Results and discussion

Study enrollment commenced in May 2019; 913 dogs were screened for study participation to meet the enrollment goal of 800 dogs. Enrollment was significantly hampered by the SARS-CoV-2 pandemic which led to cessation of non-essential research at all three study sites for variable lengths of time. Enrollment and primary vaccination of 804 dogs was completed in October 2022.

This is the first clinical study evaluating a preventative cancer vaccine based on neoantigens generated from RNA-processing errors in tumor cells. The criteria for vaccine component selection included a requirement that the antigens have human homologs. This attribute will be relevant to safety and efficacy considerations as this methodology is extended to humans. Improvements of the current vaccine for dogs is underway for evaluation in an expanded trial.

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Declaration of Competing Interest

SAJ, KFS and DHT are shareholders in Calviri Inc., which is commercializing the described frameshift vaccination technology. JLW is currently an employee of Antech Diagnostics, however this organization did not participate in funding this work. JHB, DMV, JE, JRB, LS, RLP, SA, AJ, DB, JR, IM, IDK, MKH, RH, and BMS declare they have no competing interests.

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Declaration of Generative AI and AI-assisted technologies in the writing process

No AI or AI-assisted technologies were used in the writing process.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.vetimm.2023.110691.

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