

PROTOCOL FOR ANIMAL USE & CARE

Protocol #: 19733
Date Expires: [REDACTED]

1. Contacts

Primary Investigator		Alternate Contact	
Name:	[REDACTED]	Name:	[REDACTED]
E-mail:	[REDACTED]	E-mail:	[REDACTED]
Department:	[REDACTED]	Department:	[REDACTED]
Telephone:	[REDACTED]	Telephone:	[REDACTED]
After Hours:	[REDACTED]	After Hours:	[REDACTED]

**Primary contact for sick animals*

2. Title

Phase I Study of PLZ4 decorated VBL-NP in cancer bearing dogs

3. Protocol Type

Research

[REDACTED] Clinical Trial

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[DocUpload-01-24917-\(client consent\)_ClinicalTrial.doc](#)

4. Species

Common Names	Total Number for Study	Name of Source of the Animals
dog	15	[REDACTED]

USDA: No

Detrimental Species: No

5. Brief Summary of Procedures

Dogs will be administered a nanoformulation of vinblastine (VBL-NP-PLZ4) labeled with a tumor targeting ligand once every two weeks, as long as their cancer is responding to treatment. The dose escalation of VLB-NP-PLZ4 will be performed in planned cohorts of dogs until the maximally tolerated dose is determined. Dogs will have a complete blood count, biochemical profile and urinalysis performed prior to each treatment and one week after the first treatment to monitor for side effects and guide the dose escalation.

6. Animal Location(s)

Study Area/Laboratory:

Location/Building - Room

None/Animals Will Not Leave Animal Facility

Overnight Housing (vivarium):

Vivarium(s)

Animals will be maintained by:

Vivarium

7. Special Husbandry Requirements:

No special husbandry requirements as these dogs are patients of the [REDACTED] [REDACTED] and will be treated in the same manner

as any other [REDACTED] patient.

8. Hazardous Materials:

Yes

Type	Material	Location
Hazardous Chemicals	nanoformulation of vinblastine chemotherapy	Vivarium

9. Special Procedures and/or Activities:

Use of Non-Pharmaceutical Grade Drugs

Justification for the Use of Non-Pharmaceutical Grade Drugs:

There is no pharmaceutical grade formulation of VBL-NP-PLZ4. The drug is manufactured in the [REDACTED] facility by a [REDACTED] clinical pharmacologist and is tested for potency (must be within 10% of target concentration), endotoxin and sterility prior to use. Product name, instructions regarding reconstitution, lot number and manufacturing date are included on the label and the drug stored at 4C until the day of administration. Once reconstituted, the drug will be kept at 4C until use and any drug not used within 24 hours of reconstitution will be discarded; an expiration date and time will be written on the label once reconstituted. The IACUC policy regarding the Use of Non-Pharmaceutical-Grade Compounds in Animals will be followed.

10. Funding Source(s):

Veterans Administration

11. Veterinary Care:

[REDACTED]

12. Objectives and Significance:

Objectives:

The objectives of this study are to 1) determine the recommended Phase II dose (RP2D) for future trials in dogs, 2) assess of tolerability and toxicity, and 3) gain a preliminary assessment of efficacy of VBL-NL-PLZ4, a nanoformulation of vinblastine labelled with a tumor targeting ligand.

Significance:

The objective is to provide preliminary data regarding the safety and efficacy of a nanoformulation (micelle) of vinblastine that has been labelled with a tumor targeting ligand. Vinblastine is a chemotherapy agent widely used for treatment of many human and veterinary cancers, yet acute and chronic toxicities limit the dosage of drug that can be administered during the course of treatment. Nanoparticles, like micelles and liposomes, have been investigated as alternative carriers of chemotherapy drugs as a means to decrease toxicity and improve efficacy of antineoplastic agents. Micelle formulations of chemotherapy drugs are about 20nm; this small size may increase tumor permeability resulting in increased efficacy. Micelle encapsulation of vinblastine also prevents drug release while in circulation, which will likely decrease acute side effects to the patient. PLZ4 is a molecule that has been demonstrated to have specific binding for bladder cancer and it can be linked to the surface of the micelle to allow greater concentration of the vinblastine at the tumor site as well. Identification of less toxic and more effective nanoformulations of chemotherapy drugs would allow for better long-term outcomes for people and companion animals with cancer as well as decreasing the risk of debilitating chronic side effects. Once a safe dose of VBL-NP-PLZ4 has been identified, we plan to further investigate the efficacy of this drug against bladder tumors in both dogs and eventually people as well.

13. The 3 R's - Refinement, Replacement, and Reduction:

a) Database Search for Alternatives:

1) Does this project involve USDA covered species? **No**

b) Refinement:

Collection of a voided urine sample is certainly an alternative to cystocentesis and will be utilized as a first line collection method; cystocentesis will only be performed if voided urine sample can not be collected.

c) Has this study been previously conducted?

No

d) Replacement (Species Rationale):

Vinblastine is a chemotherapy agent that is used commonly in dogs as treatment for relapsed lymphoma, histiocytic sarcoma, mast cell tumors and carcinomas, particularly transitional cell carcinoma. As preliminary, preclinical assessments of efficacy and safety of VBL-NP-PLZ4 have already been conducted, assessment in dogs with cancer for which vinblastine chemotherapy is recommended is warranted. Discovery of formulations of vinblastine that are more effective with less toxicity have the potential to improve the outcome (survival) and quality of life of dogs undergoing cancer-specific treatment.

e) Reduction (Animal Numbers Justification):

In order to minimize the number of dogs that may receive treatment with an ineffective, yet safe, dose of VBL-NP-PLZ4, an accelerated dose escalation study is proposed. In this accelerated dose escalation scheme, a single dog per dose cohort will be enrolled until a grade 2 or higher adverse event is identified as assessed using Veterinary Cooperative Oncology Group-Common Terminology Criteria for Adverse Events v1.1 (Vet Comp Onc, 2011). Once a single dog experiences a grade 2 or higher toxicity, a 3+3 phase I study design will be implemented and dose escalation rules based on three (3) dog cohorts will be used to define a well-tolerated dose. Escalation will be based on assessment of a DLT, defined as any grade 3 non-hematologic or grade 4 hematologic toxicity. There will be a two (2) week observation period between cohort escalations.

The number of dogs listed below is an estimate as to how many will be needed to completed this dose finding study. It is possible that the maximally tolerated dose will be identified with fewer than 15 dogs, at which point the study would be complete and no further dogs would be enrolled.

f) Study Groups and Numbers Table:

Group	Species	Number of Animals	Procedures/Treatments
VBL-NP-PLZ4	dog	15	All dogs enrolled will be administered the study drug

14. Procedure Details**a) Describe the use of animals in your project.**

This study is for [REDACTED] dogs diagnosed with lymphoma, mast cell tumor, histiocytic sarcoma or carcinoma. [REDACTED] dogs diagnosed with these cancers who have declined or failed standard of care treatments will be offered participation in this clinical trial. Prior to consideration to enrollment into the study, the dogs must have a physical examination, complete blood count serum biochemistry (via venipuncture) and urinalysis (via free catch or cystocentesis) within 7 days of study initiation to assess eligibility. Staging tests such as thoracic radiographs and/or abdominal ultrasound will be performed based on the supervising clinician's recommendation. Day 0 is defined as the day that the dog receives the 1st treatment with VBL-NP-PLZ4.

Day 0: [REDACTED] will complete a Quality of Life Assessment for their dog. A physical examination and tumor measurements performed using calipers, if externally measurable. An appropriately sized catheter will be aseptically placed in a peripheral vein and dogs will be administered VBL-NP-PLZ4 as a short bolus (<2 minutes). Once the infusion of VBL-NP-PLZ4 is completed, the intravenous catheter will be removed and a bandage placed at the site.

Day 7: [REDACTED] will complete a Quality of Life Assessment for their dog. A physical examination and tumor measurements performed using calipers, if externally measurable. Blood (3ml) will be collected via venipuncture of a peripheral vein and submitted for a CBC and chemistry panel. Urine will be collected either by free-catch or by cystocentesis for a urinalysis. If cystocentesis is needed to collect the urine sample, the dog will placed in dorsal recumbancy and an appropriately sized needle attached to a syringe will be inserted into the urinary bladder using ultrasound guidance.

Day 14: [REDACTED] will complete a Quality of Life Assessment for their dog. A physical examination and tumor measurements performed using calipers, if externally measurable. Blood (3ml) will be collected via venipuncture of a peripheral vein and submitted for a CBC and chemistry panel. Urine will be collected either by free-catch or by cystocentesis for a urinalysis. If cystocentesis is needed to collect the urine sample, the dog will placed in dorsal recumbancy and an appropriately sized needle attached to a syringe will be inserted into the urinary bladder using ultrasound guidance. If dogs are tolerating their treatment and their tumor is stable in size or smaller, an IVC will be aseptically placed and a 2nd dose of VBL-NP-PLZ4 will be administered as a short bolus as described for day 0.

Day 28: [REDACTED] will complete a Quality of Life Assessment for their dog. A physical examination and tumor measurements performed using calipers, if externally measurable. Staging tests such as thoracic radiographs and/or abdominal ultrasound may be recommended by the supervising clinician and performed as standard within the [REDACTED]. [REDACTED] Blood (3ml) will be collected via venipuncture of a peripheral vein and submitted for a CBC and chemistry panel. Urine will be collected either by free-catch or by cystocentesis for a urinalysis. If cystocentesis is needed to collect the urine sample, the dog will placed in dorsal recumbancy and an appropriately sized needle attached to a syringe will be inserted into the urinary bladder using ultrasound guidance. If dogs are tolerating their treatment and their tumor is stable in size or smaller, an IVC will be aseptically placed and a 3rd dose of VBL-NP-PLZ4 will be administered as a short bolus as described for day 0.

Day 42: [REDACTED] will complete a Quality of Life Assessment for their dog. A physical examination and tumor measurements performed using calipers, if externally measurable. Blood (3ml) will be collected via venipuncture of a peripheral vein and submitted for a CBC and chemistry panel. Urine will be collected either by free-catch or by cystocentesis for a urinalysis. If cystocentesis is needed to collect the urine sample, the dog will be placed in dorsal recumbancy and an appropriately sized needle attached to a syringe will be inserted into the urinary bladder using ultrasound guidance. If dogs are tolerating their treatment and their tumor is stable in size or smaller, an IVC will be aseptically placed and a 4th dose of VBL-NP-PLZ4 will be administered as a short bolus as described for day 0.

Day 56 and every 2 weeks after that time: [REDACTED] will complete a Quality of Life Assessment for their dog. A physical examination and tumor measurements performed using calipers, if externally measurable. Blood (3ml) will be collected via venipuncture of a peripheral vein and submitted for a CBC and chemistry panel. Urine will be collected either by free-catch or by cystocentesis for a urinalysis. If cystocentesis is needed to collect the urine sample, the dog will be placed in dorsal recumbancy and an appropriately sized needle attached to a syringe will be inserted into the urinary bladder using ultrasound guidance. If dogs are tolerating their treatment and their tumor is smaller in size as compared to prior to starting treatment, an IVC will be aseptically placed and a 2nd dose of VBL-NP-PLZ4 will be administered as a short bolus as described for day 0. Dogs that only have stable disease will be removed from the study at this time.

After day 56 of the study, VBL-NP-PLZ4 will be provided on a compassionate use basis for dogs that experience a partial or complete tumor response to the drug if the supervising clinician recommended further treatment with VBL-NP-PLZ4. All costs except the the cost of the drug will be assumed by the owner after day 56.

Three milliliters of blood will be collected at each visit which is within the IACUC policy.
<http://safetyservices.ucdavis.edu/article/blood-volumes>

The starting dosage of PLZ4-VBL-NP in the first cohort will be 1.0 mg/m² IV with planned escalations to 1.5 mg/m², 2.1 mg/m², 2.8 mg/m² and 3.5 mg/m². Further dose escalation will be considered based on the tolerability of 3.5 mg/m². Dose de-escalations may be considered if needed to better refine the MTD.

An accelerated dose escalation scheme will initially be performed and a single dog per dose cohort will be enrolled until a grade 2 or higher adverse event is identified. Once a single dog experiences a grade 2 or higher toxicity, a 3+3 phase I study design will be implemented and dose escalation rules based on three (3) dog cohorts will be used to define a well-tolerated dose. Escalation will be based on assessment of a DLT, defined as any grade 3 non-hematologic or grade 4 hematologic toxicity. There will be a two (2) week observation period between cohort escalations. Once the 3+3 phase is entered, the first two dogs in any cohort can be enrolled within two weeks of one another, but the 3rd dog or any other subsequent dog in the cohort must have a two-week waiting period after the previous dog is enrolled.

1. A single dog will be enrolled in the 1st cohort; if AEs are observed but less than grade 2 in severity, the dose will be escalated.
2. Once a grade 2 or high AE is observed in the single dog cohort, then the dose cohort will be expanded to 3 dogs.
3. If DLT is seen in 0/3 dogs, the dose will be escalated.
4. If DLT is seen in 1/3 dogs, an additional (up to) five dogs will be enrolled at that prescribed dose. If no DLT are seen with the additional five dogs (DLT 1/6), escalation may continue to the next higher dose.
5. If DLT is seen in 2 or more dogs (2/2, 2/3, 2/4, 2/5 or 2/6) dogs within a group, the MTD will be defined as the dose administered in the cohort below.

Dose limiting toxicities are defined as any grade 3 or higher non-hematologic and any grade 4 or higher hematologic toxicity, as defined by the Veterinary Cooperative Oncology Group - Common Terminology Criteria of Adverse Events (VCOG-CTCAE) v1.1 (Vet Comp Onc, 2011).

There is no pharmaceutical grade formulation of VBL-NP-PLZ4. The drug is manufactured in the [REDACTED] facility by a [REDACTED] and is tested for potency (must be within 10% of target concentration), endotoxin and sterility prior to use. Product name, instructions regarding reconstitution, lot number and manufacturing date are included on the label and the drug stored at 4C until the day of administration. Once reconstituted, the drug will be kept at 4C until use and any drug not used within 24 hours of reconstitution will be discarded; an expiration date and time will be written on the label once reconstituted. The IACUC policy regarding the Use of Non-Pharmaceutical-Grade Compounds in [REDACTED]

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					veterinarian [redacted] [redacted] clinical trials setting.
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted] overseeing care of patients in this study.
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	Clinical Trials Coordinator for [redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted] overseeing care of patients in this study.
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted] a veterinarian [redacted] overseeing care of patients in this study.
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted] been a veterinarian since [redacted] [redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted] a veterinarian [redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted] veterinarian [redacted] [redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted] veterinarian [redacted] [redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	Board certified medical [redacted]

Secondary Review

PI	STATION	FUNDING SOURCE	APPLICATION TITLE
[REDACTED]	Pleasant Hill, CA - 612	Veterans Affairs	Phase I Study of PLZ4 decorated VBL-NP in cancer bearing dogs

ACTION NEEDED BY IACUC

The IACUC must review the concerns listed below and decide what response is needed. This action must be documented in the IACUC minutes and the changes required by the IACUC must be incorporated into the ACORP(s) and the revised ACORP(s) must be forwarded to the CVMO for archiving.

In case of questions about this review, please contact [REDACTED], Assistant Chief Veterinary Medical Officer at [REDACTED]

REVIEWER FEEDBACK

ACORP Item number(s)	Comments/Concerns
ACORP (dog)	This protocol uses client-owned dogs with a cancer diagnosis to determine the maximally tolerated dose (MTD) of a nanoformulation of vinblastine (VBL-NP-LPZ4). Nanoparticle formulations of chemotherapy drugs may decrease toxicity and increase efficacy, which would improve the long-term outcome for both human and canine patients. The goal is to identify a safe dose of VBL-NP-PLZ4, which will then be used to evaluate its efficacy against urinary bladder tumors in dogs and eventually human patients. Dogs will be treated on an out-patient basis at the University of California-Davis (UCD) Small Animal Clinic. The principal investigator is an experienced MD genitourinary oncologist and will be supported by a large group of veterinarians specializing in oncology. Commendable aspects of the protocol include collection of voided urine samples whenever possible as opposed to cystocentesis collection and the offering of continued VBL-NP-LPZ4 treatment (after day 56) to owners whose dogs had a positive response to the drug. A few aspects of protocol should be clarified. An appendix to this review provides additional information for the IACUC's consideration. The specific numbered comments provided below must be reviewed by the IACUC, to determine what responses are needed. These actions must be documented in the IACUC minutes, and the changes required by the IACUC must be incorporated into the ACORP and the revised ACORP provided to the CVMO for archiving.
Item 6	Please consider adding a note that client-owned dogs will be treated with VBL-NP-LPZ4 at the UCD-Small Animal Clinic but will not be housed there.
Item 13	Item a) 1) – This item asks – Does this project involve USDA covered species”; the response was “No.” Based on an ILAR article entitled “Regulatory Issues Surrounding the Use of Companion Animals in Clinical Investigations, Trials, and Studies” (https://academic.oup.com/ilarjournal/article/44/3/191/678028), the dogs used in this protocol would be considered a regulated species. The article states “Animal clinical research, vaccine and drug testing, and the regulation and monitoring of clinical trials

(cont.)

	<p>may be subject both to regulation from various federal government departments and to institutional-specific policies that may address issues not completely covered by federal rules. The subject of the complex regulations related to laboratory animals is described in a previous issue of ILAR Journal (VandeBerg et al. 1999). In short, animal research of any kind is regulated by rules promulgated by the USDA in response to the AWA.” Given this information, please change the response to “Yes”, indicate and list the category D procedures that will be performed, and also conduct a literature research to consider alternatives and to prevent unnecessary duplication.</p> <p>Item e) In this item, the investigator attempts to explain how an accelerated dose escalation scheme works but a number of the terms used should be clarified to improve understanding:</p> <ul style="list-style-type: none"> • Please describe each grade of this classification system so it is clear what constitutes a grade 2, grade 3 non-hematologic or grade 4 hematologic toxicity adverse events. • Please explain the 3+3 phase 1 study design and the advantages of this design (See: https://moffitt.org/media/1310/200.pdf), providing this information will help explain why 15 dogs may be needed. Consulting a biostatistician is encouraged. • DLT is presumed to refer to dosing limiting toxicity, please address.
Item 14	The investigator refers to the IACUC policy regarding the Use of Non-Pharmaceutical-Grade Compounds in Animals; it would be worthwhile to include this policy as an attachment or briefly summarize the policy.
Item 16	In the unlikelihood event that one of the dogs is euthanized by an overdose of pentobarbital, please indicate how death will be confirmed.

Appendix – Additional Suggestions for Improvement

Comment 1:

Part 13.- Section d: The species rationale could be strengthened considerably*. (See attached example of species justification)

Comment 2:

This protocol would be easier for the general public to understand and appreciate if the following were done:

- 1) Include a section at the beginning using lay language that explained the rationale and ultimate goal of the study (including future human clinical trials?), the relevance to Veterans health, and why dogs are the appropriate choice*.
- 2) Include a section that explains the experimental design in lay language*.

Researchers often find it difficult to write in lay language, please see the attached lay language examples.

Comment 3:

Part 18 (Roster): All members of this protocol should complete the CITI Program course “**Working with Dogs in Research Settings**” or an equivalent course before beginning work on this protocol.

_____ can provide assistance with the literature search, lay language, and species justification. She can be reached _____

Example ACORP sections:

Example parts B and C1 (used with permission of the PI)

Proposal Overview

B. Description of Relevance and Harm/Benefit Analysis. Using non-technical (lay) language understood by a senior high school student, briefly describe how this research project is intended to improve the health of people and/or other animals, or otherwise to serve the good of society, and explain how these benefits outweigh the pain or distress that may be caused in the animals that are to be used for this protocol.

► Melanoma is the most dangerous form of skin cancer and kills over 9,300 people a year in the US. (see <https://www.melanoma.org/understand-melanoma/what-melanoma/melanoma-facts-and-stats> accessed 3/27/18). The main cause of melanoma is the skin being exposed to too much ultraviolet light from the sun, leading to DNA damage and cancer. Veterans have a high rate of melanoma and have additional risks than the general population because many of them served in places closer to the equator than most of the US (such as Iraq and Vietnam) where they were exposed to high levels of ultraviolet light. Melanoma is now the fifth most commonly diagnosed cancer among Veterans (see <https://www.ncbi.nlm.nih.gov/pubmed/22730846> accessed on 3/27/18)

Unfortunately, once melanoma spreads to distant sites it is usually incurable. However, new treatments that activate the patient's own immune system (immunotherapy) to fight the melanoma have worked very well in some patients. We are developing a treatment along these lines that we think will work well in many or even most patients.

Melanoma is also the most common oral cancer in pet dogs. Like human melanoma, it is an aggressive cancer that spreads to lymph nodes, lungs, liver, brain, and kidney. Despite advances in standard-of-care therapies (e.g., surgery, radiation and chemotherapy), the average survival in dogs with melanoma is less than one year after diagnosis, and less than 6 months if the melanoma has already spread. Therefore, as in human melanoma, new treatments for canine melanoma are needed to improve survival in pet dogs.

The primary goal of this study is to determine whether our new treatment is safe and has antitumor activity in canine melanoma. This is important because this treatment may let dogs with melanoma live longer or even be cured, and because canine melanoma provides a model for human melanoma because it is similar to human melanoma. The information from this study will guide further clinical trials in both dogs and humans.

C. Experimental Design.

a. Lay Summary. Using non-technical (lay) language understood by a senior high school student, summarize the conceptual design of the experiment in no more than one or two paragraphs.

► Pet dogs with melanoma will be recruited for the study by the University of Anystate Veterinary Care (UAVC) oncology service. The dog's owner will be given information about standard-of-care treatment options (including surgery, chemotherapy, radiation therapy, and commercial vaccine therapy) and will be offered participation in this study. Healthy pet dogs without melanoma visiting UAVC for routine preventive care will be recruited by the staff for collection of a blood sample for comparison to the dogs with melanoma. The owner must provide written, informed consent prior to enrolling the dog in the study.

(cont.)

Part 1 of this study tests a new immunotherapy drug called “ABC” that is injected directly into the tumor, where it will stimulate the immune system to attack the cancer cells. This drug has already been tested in children with a cancer of nerve cells (neuroblastoma) and in adults with melanoma where it was given intravenously. We will test three doses in the dogs, with the highest dose being equivalent to the dose used in the pediatric neuroblastoma study. At each dose we will take blood samples and tumor biopsies from the dogs to see how well the drug activated their immune systems, and we will monitor the dogs for any side effects from the drug.

Part 2 will use the best dose of ABC from part 1, combined with radiation therapy directed at the tumor being injected with ABC. Radiation therapy is a standard treatment for dogs with melanoma, but there are two ways to do it: 1) Giving the radiation all at once or 2) Giving a higher dose of radiation, but spread over three days with two days in between treatments. We will test both ways of giving the radiation, take blood samples and tumor biopsies to determine which radiation therapy results in the stronger immune system activation, and monitor for side effects.

Part 3: We know tumor cells produce substances that inhibit the immune system, so we will add a drug called “XYZ” which will prevent the immune system from being inhibited. This drug will be combined with the best ABC dose from part 1 and the best way of giving the radiation from part 2. There will be two groups of dogs:

Group 1: Dogs with melanoma that has not spread very far. The primary tumor will be injected with ABC and treated with radiation and all remaining sites of tumor will also be treated with radiation. The XYZ will be given intravenously so it goes all over the body.

Group 2: Dogs with melanoma that has spread far so it is not feasible to treat all the tumors with radiation. The primary tumor will be treated with ABC and radiation, and the XYZ will be given intravenously so it goes all over the body. If successful, the immune system will be activated so it goes and attacks even the untreated tumors.

The overriding goal of this canine clinical trial is to evaluate this new combination treatment for melanoma in large animals (pet dogs) before testing this treatment in people. The main thing we want to know is whether this combination treatment is safe enough for testing in people, but we will also look at how well the immune system gets activated, and how much the tumors shrink.

Example Part D - Species. Justify the choice of species for this protocol.

► Pet dogs develop melanoma spontaneously, and like human patients are of various ages, mixed gender, and share similar environmental exposures. Human and canine melanoma are very similar in that both cancers have a lot of mutations; the same drugs will work for some people and dogs but not others; and the melanoma cells of both species share certain cancer molecules. Many trials of treatments for melanoma in dogs have led to successful treatments for people.

Unfortunately the pig model for spontaneous melanoma (Sinclair swine melanoma model) does not appear to be as good as the dog model to study this treatment approach. No one has tested whether hu14.18-IL2 will work on melanoma in pigs, but we already know it can have an effect on melanoma in dogs and people. Furthermore, there is no anti-PD1 antibody available for pigs, and the ones for humans and dogs won’t work in pigs.

Mice are often used for cancer studies, but melanoma in mice is quite different from melanoma in dogs and people. As a result, melanoma treatments that work in the mice usually don’t work in people. In contrast, treatments that work in dogs more often can work in people. Thus, this study will help improve melanoma treatment for both species.

(cont.)

Example Part W dog cancer ACORP

A. Consideration of Alternatives and Prevention of Unnecessary Duplication Minimize harm derived from the proposed work. Document the required efforts to “Replace, Reduce, Refine” and searches conducted.

1. **List each** of the potentially painful or distressing procedures included in this protocol.
 - ▶ Diagnosis of spontaneous melanoma
 - ▶ Tumor biopsies
 - ▶ Thoracic radiograph
 - ▶ Radiation therapy treatment
 - ▶ Cancer therapy adverse events

Document database search(s) in the table below. Then answer Items W.2 through W.5 regarding potentially painful or distressing procedures.

Name of database	Date of search	Years covered by the search	Potentially painful or distressing procedures addressed	Key words and/or search strategy used	Indicate which mandate each search addressed			
					Replacement of animals (item W.2)	Reduction in animal numbers used (W.3)	Refinement to minimize pain or distress (W.4)	Lack of unnecessary duplication (item W.5)
ALTBIB search for Citations with “Animal Use Alternatives” as the main topic.	3/6/18	All years available	melanoma diagnosis	melanoma diagnosis	(X)	()	()	()
ALTBIB citations from 2000 to present	3/6/18	2000-2018	melanoma diagnosis	Melanoma, XYZ	(X)	(X)	(X)	()
ALTBIB citations from 2000 to present	3/6/18	2000-2018	Tumor biopsy	“tumor biopsy”, dog	(X)	(X)	(X)	()
ALTBIB citations from 2000 to present	3/6/18	2000-2018	Thoracic radiograph	“thoracic radiograph”, dog	(X)	(X)	(X)	()
ALTBIB citations from 2000 to present	3/6/18	2000-2018	Radiation therapy	“radiation therapy”, dog	(X)	(X)	(X)	()

(cont.)

PubMed	3/6/18	All years available	Cancer therapy adverse events	Adverse events and ABC treatment,	()	()	(X)	()
PubMed	3/6/18	All years available	Cancer therapy adverse events	adverse events and XYZ treatment	()	()	(X)	()
PubMed	3/6/18	All years available	Cancer therapy adverse events	adverse events and radiation therapy	()	()	(X)	()
PubMed	3/6/18	All years available	Confirming lack of unnecessary duplication	canine melanoma, ABC, XYZ	()	()	()	(X)

2. **Replacement.** Describe the replacements that have been incorporated into this work, the replacements that have been considered but cannot be used, and the reason(s) that further replacements are not acceptable.

► We ran a search on the ALTBIB (Alternatives to Animal Testing) website at <https://toxnet.nlm.nih.gov/altbib.html> looking specifically for papers related to melanoma and melanoma diagnosis with “animal use alternatives” as the main topic. Three papers met the search criteria: one was about establishing a tissue bank, one used a red dye in an *in vitro* test to determine the viability of tumor cells, and one was about using confocal microscopy to look at tumors in living skin. None of these were computer models or *in vitro* models for testing new treatments for melanoma. Our study requires an intact animal with a spontaneous tumor and immune system to reach our objective, and this cannot be accomplished with computer modeling or replicated with *in vitro* tissue culture. Clinical evaluation, by definition, requires the observation of a live animal.

A second search of the ALTBIB website using “melanoma and XYZ” for alternative animal models resulted in nine papers, of which six looked at mouse models of melanoma. Although work with mice has been crucial in developing and testing new treatment approaches melanoma (including using XYZ), dogs that develop melanoma spontaneously are much closer to the human disease. Similar to human melanoma, spontaneous canine melanoma is an aggressive cancer and that spreads to distant sites such as lymph nodes, lungs, liver, brain, and kidney. Moreover, there is a disconnect between the number of anti-cancer therapeutics that work in mice versus in humans. Further, despite advances in standard-of-care therapies (e.g., surgery, radiation and chemotherapy), survival in dogs with melanoma is less than one year after diagnosis, and less than 6 months if the melanoma has spread to other sites. Two papers were *in vitro* studies, which as noted above do not replicate an intact immune system or the distant melanoma metastases our study requires. One paper examined genetic and protein mutations from melanoma samples, in concert with The Cancer Genome Atlas (TCGA) database, to form insights to the treatment of melanoma.

As noted above in the species justification section (section D) pigs are not suitable for this study because pigs do not develop spontaneous melanoma as dogs do. Further, it is not known whether porcine melanomas express the GD2 antigen targeted by ABC.

Finally, we want to point out this study will lead to an improved treatment of melanoma for both people and dogs, which makes pet dogs that spontaneously develop melanoma the most appropriate study subjects.

3. **Reduction.** Describe how the number of animals to be used has been minimized in this protocol and explain why further reduction would disproportionately compromise the value of the data.

► We have worked with our collaborator Dr. XXX, biostatistician at the University of Anystate to determine the minimum number of animals to be used. Please see section C2b for details.

(cont.)

4. **Refinement.** Describe the refinements that have been incorporated into this work and explain why no further refinements are feasible.

► An ALTBIB search for alternative methods for tumor biopsy in dogs produced only two papers. The methods described are the same methods/standard of care utilized at UA Veterinary Care. An ALTBIB search for alternative methods for thoracic radiographs in dogs did not produce any papers. We routinely run thoracic radiographs on dogs at the UA Veterinary Care oncology clinic using standard of care for pet dogs. An ALTBIB search for alternative methods for radiation therapy in dogs produced 11 papers. Several papers examined *in vitro* model using cell lines, whereas others combined radiation therapy with other modalities. The dose and schedule of radiation proposed in this study are standard of care for dogs with spontaneous melanoma. Moreover, our collaborator Dr. XXX, DVM, is a board certified veterinary radiation oncologist and medical oncologist has prepared radiotherapy plans for this study and will oversee radiation treatments. The procedures and potential adverse events described in this protocol are either standard of care or are well known and experienced by the veterinary care staff involved. Further refinements will be incorporated as they become available and/or known to the team. We keep current in the published literature by checking PubMed for updates and/or alternatives to procedures used.

5. Describe how it was determined that the proposed work does not unnecessarily duplicate work already documented in the literature.

► A PubMed search for the following keywords: melanoma, ABC, XYZ failed to produce any publications. Our proposed study is original work in a cutting-edge area of cancer research and work like this has not been published before.