ANIMAL COMPONENT OF RESEARCH PROTOCOL (ACORP)
Main Body

[redacted] *new protocol for JIT*

A. ACORP Status.

1. Full Name of Principal Investigator(s)► [redacted]**, MD**
2. VA Station Name and 3-Digit Station Number► **William S. Middleton Memorial VA Hospital, site 607**
3. Protocol Title► **Administration of intratumoral immunocytokine to activate immune rejection of spontaneous canine melanoma**
4. Animal Species covered by this ACORP► ***Canis familiaris***
5. Funding Source(s). Check each source that applies:
   ►(**X**) Department of Veterans Affairs.
   ►( ) US Public Health Service (e.g. NIH).Include grant title, grant #, & UW WISPER #:
   ►( ) Private or Charitable Foundation -- Identify the Foundation:
   ►( ) University Intramural Funds – Identify the University and Funding Component:
   ►( ) Private Company – Identify the Company:
   ►( ) Other – Identify Other Source(s):
6. Locations where proposed animal and laboratory work will occur:

Laboratory Space		Animal Housing			Animal Procedures		Transporting Animals to VA Lab Space		
VA	UW	VA	UW	N/A	VA	UW	Yes	No	N/A
( )	(**X**)	( )	( )	(**X**)	( )	(**X**)	( )	(**X**)	( )

7. Related Documentation for IACUC reference.
   a. New protocol submission. If this protocol applies to a project that has already been submitted to the R&D Committee for review, identify the project:
      (1) Title of project► **N/A**
      (2) If approved by the R&D Committee, give the date of approval► **N/A**
   b. Triennial review. If this protocol is being submitted for triennial *de novo* review, complete the following:
      (1) Identify the studies described in the previously approved ACORP that have already been completed.
      ►**N/A**
      (2) Indicate the numbers of animals of each breed/strain/genotype that have already been used, and adjust the numbers shown in Item I accordingly.
      ►**N/A**
      (3) Describe any study results that have prompted changes to the protocol, and briefly summarize those changes, to guide the reviewers to the details documented in other Items below.
      ►**N/A**
   c. List any other relevant previously approved animal use protocols (copy below as needed).
      (1) Title of other protocol ►**Phase I Dose-Finding Trial of IT-IC in Tumor-Bearing Dogs**
      (2) IACUC approval number of other protocol ► [redacted]
      If not approved by **site 607**, provide the VA station or other approving institution ►**University of Wisconsin School of Veterinary Medicine (UW-SVM)**
8. Indicate the type(s) of animal use covered by this protocol (check all that apply):

►(**X**) Research
►( ) Teaching or Training
►( ) Testing
►( ) Breeding and colony management only; not for any specific research project
►( ) Holding protocol (as specified by local requirements; not required by VA, PHS, or USDA)
►( ) Other. Please specify►

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. 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.

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 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.

1. **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 Wisconsin Veterinary Care (UWVC) 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 UWVC 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.

Part 1 of this study tests a new immunotherapy drug called "hu14.18-IL2" 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 hu14.18-IL2 from part 1, combined with radiation therapy directed at the tumor being injected with hu14.18-IL2. Radiation therapy has been used for nearly a century to treat human cancers, and radiation therapy is a standard treatment for dogs with melanoma. Radiation can cause the tumor to shrink, activate the immune system, and may also reduce or eliminate other cells that inhibit the body's immune system. There are two ways to deliver radiation therapy: 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. Radiation will be given to the dogs using state-of-the-art equipment, similar to what would be used for a human. 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 "anti-PD1 antibody" which will prevent the immune system from being inhibited. This drug will be combined with the best hu14.18-IL2 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 hu14.18-IL2 and treated with radiation and all remaining sites of tumor will also be treated with radiation. The anti-PD1 antibody 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 hu14.18-IL2 and radiation, and the anti-PD1 antibody 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.

2. **Complete description of the proposed use of animals.** Detail the proposed use of animals:

   a. **Summarize** the design of the experiment in terms of the specific groups of animals to be studied.

   ► The primary goal of this study is to determine whether intratumoral immunotherapy with hu14.18-IL2 (IT-IC) in combination with local radiation therapy (RT) and immune checkpoint blockade is safe and has antitumor activity in canine melanoma. Dogs in the proposed study will be privately owned pets with spontaneously occurring melanoma. Exploratory studies will: 1) evaluate T cell responses in the blood and tumor before and after this immunotherapy, and; 2) utilize novel immune monitoring to identify a candidate biomarker of response for dogs with melanoma receiving IT-IC. In addition, blood samples will be collected from healthy pet dogs that do not have melanoma to be used as controls for flow cytometry and T cell receptor (TCR) repertoire analyses.

   Part 1 (Aim 1a): We will initially study a low, medium, and high dose of IT-IC in 9-18 dogs with locally advanced or metastatic melanoma and will identify a maximum tolerated dose (MTD) or maximum administered dose (MAD) of IT-IC using a 3-day administration schedule. The doses used will not exceed that found to be safe in human pediatric neuroblastoma patients.

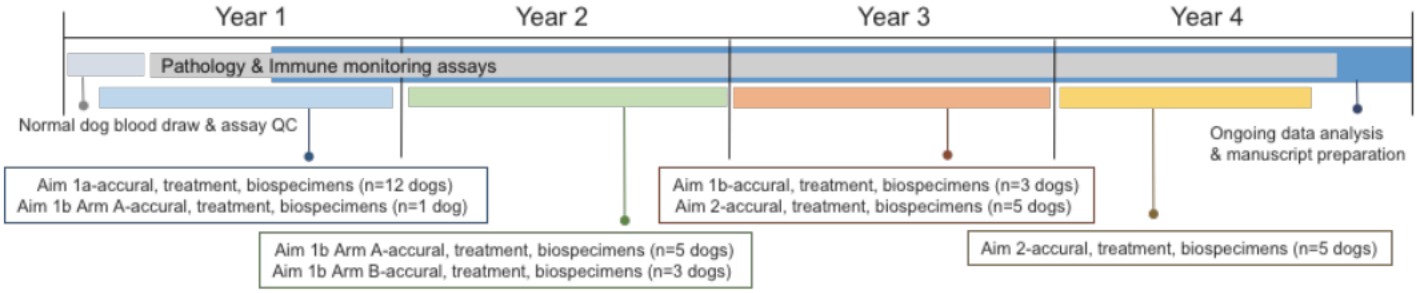
   We recently completed preliminary safety testing, using separate funds, of the first two proposed dose levels of hu14.18-IL2 in tumor-bearing dogs at UWVC (Protocol Title: "Phase I Dose-Finding Trial of IT-IC in Tumor-Bearing Dogs", IACUC approval number [redacted]). These doses are lower than doses previously given

intravenously to human pediatric neuroblastoma patients. The treatment was well tolerated by the treated dogs, however, only safety data, not biological data, were collected. Therefore, we will describe the study as designed but will allow for modification of cohorts and/or doses once the study is approved.

Part 2 (Aim 1b): Once the MTD or MAD is determined in Part 1, we will study another 12 dogs with locally advanced or metastatic melanoma to evaluate the safety of IT-IC at the MTD or MAD combined with RT to the local site, the same tumor site receiving IT-IC, in order to enhance its function as an *in situ* vaccine. The RT will be given either in a single 8 Gray (Gy) fraction or in three 8 Gy fractions (the Gray (Gy) is a derived unit of ionizing radiation dose, at the absorption of one joule of radiation energy per kilogram of matter. It is used to measure the amount of radiation delivered in this study) and we will determine whether a single 8 Gy fraction or three 8 Gy fractions of RT merit subsequent testing with IT-IC in canine melanoma.

Part 3 (Aim 2): After completion of Aim 1, we will enroll another 12 dogs with locally advanced or metastatic melanoma into Aim 2 to determine safety, tolerability, and antitumor activity (based on clinical measurements as well as histological data) of the combination of local RT, IT-IC and anti-PD1 antibody. We will administer a caninized anti-canine-PD1 antibody (hereto referred to as 'anti-PD1') that has been designed to be recognized as "self" by the canine immune system. We will evaluate mechanisms of antitumor activity and will determine whether histologic findings of concomitant immune tolerance seen in our murine model are also present in the dog.

Blood samples and tumor biopsies will be obtained in Aims 1 and 2 for immune monitoring in Aim 3. This study has potential for high clinical impact as study findings in the dog will inform clinical development of IT-IC in human melanoma patients.



b. **Justify the group sizes and the total numbers of animals requested.** A power analysis is strongly encouraged; see ACORP instructions.

► Dr. [redacted], biostatistician collaborator on this VA Merit grant, has reviewed the study design. Since the study was originally designed and submitted, we have obtained pilot safety data from a Phase I dose-escalation study (IACUC approval number [redacted]). However, as biologic data was not collected in the Phase I study, the exact effect of the candidate biomarkers are not known. The primary outcome for the dose finding parts of the study is toxicity, whereas the primary outcome for the subsequent parts of the study is the determination of candidate biomarkers of response to the treatment.

The planned sample size for this study is between 38-47 dogs with melanoma (Part 1, Aim 1a: 9-18 dogs; Part 2, Aim 1b: 12 dogs; Part 3, Aim 2: 12 dogs); and 5 pet control dogs.

- Part 1 (Aim 1a).
  A total of 9-18 dogs (estimate 12 dogs) will be enrolled in this Aim. As this is a dose escalation study with the primary objective to determine the MTD/MAD of IT-IC when given daily for 3 days to dogs with canine melanoma, no formal power calculations were conducted. Rather, the sample size chosen is based on a typical "3+3" dose escalation schema with 3 planned for each dose cohort and expansion to 6 dogs when indicated. The total number of dogs treated for this part of the study will depend on the number of dogs treated in each cohort before the MTD/MAD has been determined. It is expected that a total of

approximately 12 dogs (9-18) will be required to complete the dose escalation. Information from the tumor-bearing dogs in the separate study (Protocol Title: "Phase I Dose-Finding Trial of IT-IC in Tumor-Bearing Dogs", IACUC approval number [redacted]) will also be used to make decisions about the MTD/MAD of IT-IC for this Aim.

- Part 2 (Aim 1b).
  A sample size of 6 dogs per treatment group (total of 12 dogs) will be enrolled in this Aim. This sample size will be adequate to detect anticipated moderate to large effect sizes with sufficient power when comparing candidate biomarker levels between arms. Specifically, a sample size of 6 dogs per arm will provide between 49-94% power at the one-sided 0.05 significance level to detect anticipated effect sizes ranging between 1.0-2.0 standard deviation units in candidate biomarker levels. Thus, the power would be 94% at the one-sided 0.05 significance level to detect an effect size of 2.0 standard deviation units in candidate biomarker level. Due to the exploratory nature of this study, there will be no multiple testing adjustments for evaluating multiple candidate biomarkers.

- Part 3 (Aim 2).
  A sample size of 6 dogs per treatment group (total of 12 dogs) will be enrolled in this Aim. This sample size will provide 70-99% power to detect a moderate effect size of 1.0-2.0 standard deviation units for the change in biomarker levels of T cell response to melanoma from the baseline to the RT, IT-IC, and anti-PD1 antibody post treatment assessments at the one-sided 0.05 significance level. Furthermore, large effect sizes of >2.5 for the differences of changes from baseline in biomarker levels between groups will be detected with 90% power at the two-sided 0.0167 (=0.05/3 – a Bonferroni adjustment for multiple comparisons between two groups). The study does not provide adequate power for detecting the anticipated correlations between changes in biomarker levels of T response and tumor expression of GD2 within each group. However, across the two groups, a moderately strong correlation of 0.6 or greater will be detected with 80% power at the two-sided 0.05 significance level.

c. **Describe each animal procedure** to be performed on this protocol. (Document in Appendix 9 any of these procedures that involve "departures" from the standards in the *Guide*. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)

► Information on recruitment, inclusion criteria, blood collection from controls, and pretreatment evaluations are included in Appendix 1.

Collection of Blood Samples from Dogs without Melanoma to Serve as Assay Controls

- A blood sample at one timepoint will be collected from 5 privately-owned dogs upon the owner's consent.

<u>Treatment for Pet Dogs with Melanoma</u>

- *Part 1 (Aim 1a) Schedule*: Dose escalation study to determine MTD or MAD of IT-IC.
  - This dose escalation study will include 3 dogs/cohort, but will allow for expansion of each cohort to 6 dogs/cohort if dose-limiting toxicity (DLT) is seen in one dog in the planned 3 dogs in each cohort (estimate 12 dogs total for the dose escalation part of the study). The IT-IC will be administered to the treatment site on days 1, 2 and 3. We will obtain tumor biopsies for analysis of melanoma tumor cells and tumor infiltrating lymphocytes (TIL) as well as peripheral blood mononuclear cells (PBMC) for immune monitoring at baseline and at various times post-injection. The dose levels planned for testing are based on our prior studies involving intravenous administration of hu14.18-IL2 in adults with melanoma and in children with neuroblastoma. The treatment groups to be studied are:
    - hu14.18-IL2 (2.0 mg/M2/day) x 3 days
    - hu14.18-IL2 (6.0 mg/M2/day) x 3 days
    - hu14.18-IL2 (12.0 mg/M2/day) x 3 days

- *Part 2 (Aim 1b) Schedule*: Determine localized and systemic toxicity of IT-IC at the MTD or MAD when given daily for 3 days following RT to dogs with canine melanoma.

- o The MTD or MAD of IT-IC from Aim 1a will be combined with RT in Aim 1b for dogs (6 dogs/cohort) with locally advanced melanoma. The RT will be delivered in a single 8 Gy fraction or in three 8 Gy fractions over 1 week (i.e., Day -10, Day -8, Day -6) to the primary site and regional lymph nodes when clinically involved (locally advanced melanoma) approximately 6 days or between 10 and 6 days, respectively, prior to IT-IC. The tumor will be biopsied pretreatment as well as 1, 2, and 3 weeks after IT-IC. The treated tumor will be left in place for 3 weeks in all dogs to allow it to function as an *in situ* vaccine. If dose limiting toxicity is seen in 2 dogs, 6 additional dogs would be entered at a lower dose of IC, or lower dose of RT, depending on which seemed likely as the cause for the DLT. Safety data will be reviewed and a dose reduction will be considered in the unlikely event that DLTs are observed in more than 2 of the initial 6 dogs.
- o Blood will be collected for immune monitoring pretreatment and various times post IT-IC treatment.

- *Part 3 (Aim 2) Schedule*: Determine the safety and tolerability of the combination of local radiation, systemic anti-PD1 antibody, and IT-IC in dogs with locally advanced or metastatic melanoma.
  - o The MTD or MAD of IT-IC combined with RT from Aim 1b will be combined with anti-PD1 antibody. A total of 12 dogs with locally advanced or metastatic melanoma will be studied in this Aim in one of the following 2 groups:
    - Group A: 6 dogs with locally advanced or regional melanoma, but without distant metastases will receive IT-IC + RT treatment to all sites of tumor, in combination with anti-PD1 antibody.
    - Group B: 6 dogs with locally advanced or regional melanoma, and also with distant metastases, will receive IT-IC + RT treatment to the locally advanced or regional melanoma, but no RT treatment to distant metastases, in combination with anti-PD1 antibody.
  - o We will obtain tumor biopsies for analysis of melanoma tumor cells and TIL as well as PBMC for immune monitoring at baseline and at various times post-injection.

Patient Follow-Up (Melanoma Dogs only)

- A physical examination including tumor measurements will be performed at each study visit.
- Tumor biopsies will be collected on days 10, 17, and 24. CBC, chemistry profile, and urinalysis will be performed on days 10 and 24. On days 10, 30 and 60, 12 ml of blood for immune assays will be collected and thoracic radiographs will be repeated on Day 30.

Treatment and Evaluation Schedules for Parts 1-3 (Aims 1 and 2 only; no dogs are treated in Aim 3)

Part 1 (Aim 1a):

	Pre-Treatment	Day 1	Day 2	Day 3	Day 10	Day 17	Day 24	Day 30	Day 60
Complete physical examination	X	X			X	X	X	X	
CBC, biochemistry profile and urinalysis	X				X		X		
Tumor biopsy	X				X	X	X		
Fine needle aspirate of lymph node	X								
Blood collected for *in vitro* assays	X				X			X	X
Thoracic radiographs	X							X	
IT-IC administration		X	X	X					

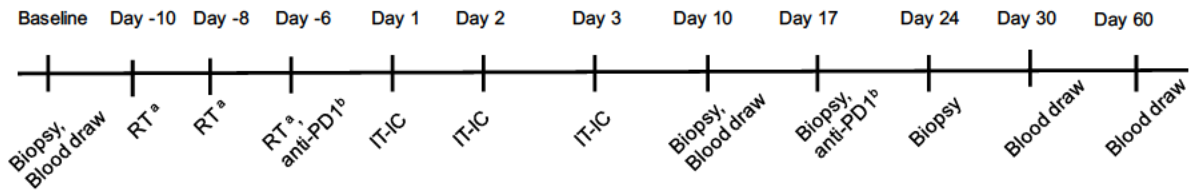
Part 2 (Aim 1b):

	Pre-Treat-ment	Day -10	Day -8	Day - 6	Day 1	Day 2	Day 3	Day 10	Day 17	Day 24	Day 30	Day 60
Complete physical examination	X			X	X			X	X	X	X	
CBC, biochemistry profile and urinalysis	X							X		X		

Tumor biopsy	X							X	X	X		
Fine needle aspirate of lymph node	X											
Blood collected for *in vitro* assays	X							X			X	X
Thoracic radiographs	X										X	
RT planning and treatment[1]		X[1]	X[1]	X[1]								
IT-IC administration					X	X	X					
[1] 3x 8 Gy fractions between Days -10, -8 and -6; or 1x 8 Gy fraction on Day -6												

Part 3 (Aim 2):

	Pre-Treat-ment	Day -10	Day -8	Day - 6	Day 1	Day 2	Day 3	Day 10	Day 17	Day 24	Day 30	Day 60
Complete physical examination	X			X	X			X	X	X	X	
CBC, biochemistry profile and urinalysis	X							X		X		
Tumor biopsy	X							X	X	X		
Fine needle aspirate of lymph node	X											
Blood collected for *in vitro* assays	X							X			X	X
Thoracic radiographs	X										X	
RT planning and treatment[1]		X[1]	X[1]	X[1]								
Anti-PD1 treatment[2]				#1					#2			
IT-IC administration					X	X	X					
[1] 3x 8 Gy fractions between Days -10, -8 and -6; or 1x 8 Gy fraction on Day -6 [2] In dogs with macroscopic metastatic disease experiencing stabilization or regression, continuation of Anti-PD1 therapy beyond the protocol activities will be offered to the owners at the owner's expense.												



Treatment Schema. All dogs will receive IT-IC on 3 consecutive days. Aim1b dogs will also receive radiation therapy (RT) to the local site: 1x 8 Gy (Arm A) or 3x 8 Gy fractions over 1 week (Arm B). Aim 2 dogs will receive the optimal RT from Aim 1b. In addition, Aim 2 dogs will receive anti-PD1 antibody (anti-PD1) every 3 weeks for 2 doses, starting on Day -6. Continuation of anti-PD1 therapy beyond the protocol activities can be continued apart from this protocol for Aim 2 dogs with macroscopic metastatic disease experiencing stabilization or regression of disease. Biopsies and blood are collected at the indicated times for histological, immune function and molecular analyses in Aim 3. RT[a], Aims 1b & 2; anti-PD1[b], Aim 2 only

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 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.

Personnel

E. Current qualifications and training. (Plans for additional training will be requested in Item F.)

1. PI Name► [redacted], **MD**
   Animal research experience ► **N/A**

Specific procedure(s) **Dr.** [redacted] will perform	Experience with each procedure in canines
Study Principal Investigator; Will not handle or treat live animals.	N/A

2. Other research personnel (copy the lines below for each individual)

Name► [redacted], **DVM, DACVIM (Oncology)**
Animal research experience ►**Has worked with dogs since 1984**

Specific procedure(s) **Dr.** [redacted] will perform	Experience with each procedure in canines
Dr. [redacted] will direct the clinical trial in this project. His activities will include identification of appropriate dogs with melanoma for this study as well as perform or oversee experimental intratumoral immunocytokine (IT-IC) delivery, anti-PD-1 antibody administration, procurement of blood, urine, fine needle aspirate of draining lymph node and tumor biopsy samples, and clinical care of companion dogs in this study.	[redacted] is a boarded veterinary oncologist who has been performing these procedures in dogs since 1984.

Name► [redacted], **DVM, DACVIM (Oncology), DACVR (Radiation Oncology)**
Animal research experience ► **Veterinarian – has worked with dogs since 1998**

Specific procedure(s) **Dr.** [redacted] will perform	Experience with each procedure in canines
Dr. [redacted] will be responsible for overseeing, planning, and delivering the radiation therapy to the companion dogs in this study.	Dr. [redacted] is a boarded veterinary radiation oncologist and has been performing these procedures in dogs since 2004.

Name► **Oncology Clinical Trial Intern** (to be determined)
Animal research experience ► **Veterinarian**

Specific procedure(s) **Clinical Trial Intern** will perform	Experience with each procedure in canines
The clinical trial intern will assist Dr. [redacted] in experimental immunocytokine (IT-IC) delivery, anti-PD-1 antibody administration, procurement of blood, urine, fine needle aspirate of draining lymph node and tumor biopsy samples, and clinical care of companion dogs in this study.	Licensed Veterinarian who will be experienced with the performance of these procedures.

Name► [redacted]**, PhD Medical Microbiology & Immunology**
Animal research experience ► **N/A**

Specific procedure(s) [redacted] will perform	Experience with each procedure in canines
Dr. [redacted] will be responsible for performing the *in vitro* functional lymphocyte assays, all molecular biology assays, and logging and storing lymphocyte samples. Will not handle or treat live animals.	N/A

Name► [redacted]**, MA, MS, EdD (College Teaching of Biology)**
Animal research experience ► **Has worked with dogs since 1979.**

Specific procedure(s) [redacted] will perform	Experience with each procedure in canines
Dr. [redacted] will be responsible for coordinating the clinical aspects of the proposed trial including scheduling subject treatments and evaluations, clinical budget management, and clinical data collection and follow-up. Will not handle or treat live animals.	N/A

Name► [redacted]
Animal research experience ► **N/A**

Specific procedure(s) [redacted] will perform	Experience with each procedure in canines
Mr. [redacted] will be responsible for processing of all tissue and blood samples from dogs enrolled in the clinical trial. Will not handle or treat live animals.	N/A

3. a. Veterinary support staff/ personnel (list AALAS certification, experience, or completion of special trainings)

At the VA
Name► [redacted]**, VMC**

Specific support procedure(s) assigned to **Dr.** [redacted]	Qualifications for performing each support procedure in canines
Animal health & Veterinary oversight	Dr. [redacted] has over 37 years of experience in the field, and has been a veterinarian at the VA Hospital for over 17 years, and is a member of both AALAS and ASLAP.

At the UW SVM
Name► [redacted]**, DVM, DACLAM**
[redacted]**; cell:** [redacted]

Specific support procedure(s) assigned to [redacted]	Qualifications for performing each support procedure in canines
Animal health & Veterinary oversight	RARC Senior Program Veterinarian (SVM)

b. **VA animal care** support staff / personnel (list AALAS certification, experience, or special trainings)

Name► N/A- no canine care at VMU

UW SVM animal care support staff / personnel (list AALAS certification, experience, or special trainings)

Name► [redacted] **, BA, MA**
Phone: [redacted]

Specific support procedure(s) assigned to [redacted]	Qualifications for performing each support procedure in canines
IACUC oversight; Will not handle or treat live animals.	IACUC Administrator; Contact for protocol submission and compliance questions

4. For research personnel in items 1 and 2 above, enter the most recent completion date for each CITIPRROGRAM.ORG course

Name of Individual	Working with VA IACUC **MM/DD/YY**	Working with Dogs in Research Settings **MM/DD/YY**	Completed RARC trainings Animal User Orientation **MM/DD/YY** Biomethodology of the Dog **MM/DD/YY**
[redacted]	04/17/18	03/07/18	Animal User Orientation 9/28/14
[redacted]	04/20/18	04/20/18	Animal User Orientation 11/14/17 Worked with dogs in clinical settings since 1984 - grandfathered by RARC
[redacted]	04/17/18	04/18/18	Animal User Orientation 4/15/15 Worked with dogs in clinical settings since 1998 - grandfathered by RARC
[redacted]	03/08/18	03/6/18	Animal User Orientation 12/21/04
Oncology Clinical Trial Intern – to be identified			
[redacted]	04/19/18	04/19/18	Animal User Orientation 12/02/17
[redacted]	04/06/18	04/06/18	Animal User Orientation 5/14/13
All personnel, including the to be identified Oncology Clinical Trial Intern, will complete required training modules and will not begin work until all mandatory training has been completed.			

F. **Training to be provided.** For each procedure from Item E for which anyone is shown as "to be trained": describe the type of training to be provided, then name(s), qualifications, and experience of the trainer(s). If no further training is required for anyone listed in Item E, enter "N/A"

► The clinical trial intern will receive one-on-one training on the proper delivery of experimental therapeutics (e.g., immunocytokine (hu14.18-IL2), anti-PD1 antibody) to dogs for this study. As a licensed veterinarian, the clinical trial intern will be experienced and knowledgeable regarding the procurement of biological specimens from dogs, as well as clinical care of dogs. Dr. [redacted], a boarded veterinary oncologist who has been performing these procedures in dogs since 1984, will provide the training.

G. **Occupational Health and Safety.**

1. Complete one line in the table below for each person identified in Item E:

Name	Occupational Health and Safety Program Coverage		Declined optional VA services	Current on Interactions with OHSP, or UWOM? (yes/no)
	VA	Equivalent Alternate Program, UW Occupational Medicine (UWOM) Program Yearly Animal Contact Risk Assessment Questionnaire (ACRQ) Requirement		
[redacted]	N/A	UW ACRQ Completed	X	yes

[redacted]	N/A	UW ACRQ Completed	X	yes
[redacted]	N/A	UW ACRQ Completed	X	yes
Oncology Clinical Trial Intern (to be identified)	N/A	UW ACRQ will be completed	TBD	TBD
[redacted]	N/A	UW ACRQ Completed	X	yes
[redacted]	N/A	UW ACRQ Completed	X	yes
[redacted]	N/A	UW ACRQ Completed	X	yes
Personnel exposed to dogs receive rabies vaccination and yearly serum rabies virus neutralization tests. TBD, to be determined upon identification of the individual for this UW position.				

2. Are there any non-routine OHSP measures that would potentially benefit, or are otherwise required for, personnel participating in or supporting this protocol?
   ► (**X**) Yes. Describe them ► **Personnel exposed to dogs receive rabies vaccination and yearly serum rabies virus neutralization tests.**

Animals Requested

H. Animals to be Used. List animals according to any specific features required for the study (ACORP Instructions provide guidance on specific terminology recommended for the "Health Status" column):

Description (special features not shown elsewhere in table)	Gender	Age/Size on Receipt	Source (Vendor, Collaborator, PI of local breeding colony)	Health Status
Privately owned companion dogs with spontaneously developed melanoma	Male & female	Will vary based on enrollment	Privately owned companion dogs	Diagnosed with melanoma
Privately owned healthy companion dogs	Male & female	Will vary based on enrollment	Privately owned companion dogs	Healthy

I. Numbers of dogs requested. See ACORP Instructions, for descriptions of the categories and how to itemize the groups of animals.

USDA Category B

Procedures► **N/A**						
Experimental Group / Procedures(s)	Year 1	Year 2	Year 3	Year 4	Year 5	Category B TOTAL

USDA Category C

Procedures► **see Appendix 4 below**						
Experimental Group / Procedure(s)	Year 1	Year 2	Year 3	Year 4	Year 5	**Category C TOTAL**
Single blood draw from healthy companion dogs	5					5
Collection of blood and urine samples from dogs with melanoma	Up to 19	8	9	6		42

USDA Category D

Procedures► **see table J below**						
Experimental Group / Procedure(s)	Year 1	Year 2	Year 3	Year 4	Year 5	**Category D TOTAL**
IT-IC Dose-finding trial	Up to 18					18
Combination IT-IC and radiation therapy trial	1	8	3			12
Combination IT-IC, radiation therapy, and Anti-PD-1 antibody trial			6	6		12

USDA Category E

Procedures► **N/A**						
Experimental Group / Procedure(s)	Year 1	Year 2	Year 3	Year 4	Year 5	**Category E TOTAL**

TOTALS over all Categories

Experimental Group /Procedure(s)	Year 1	Year 2	Year 3	Year 4	Year 5	**GRAND TOTAL**
IT-IC Dose-finding trial	Up to 18					18
Combination IT-IC and radiation therapy trial	1	8	3			12
Combination IT-IC, radiation therapy, and Anti-PD-1 antibody trial			6	6		12
Blood collection from healthy companion dogs to be used as controls for in vitro assays.	5					5
	24	8	9	6		47

J. **Management of USDA Category D procedures**. Indicate which statement below applies, and provide the information requested.

► (**X**) This protocol INCLUDES Category D procedures.
List each Category D procedure and provide information requested.
(For surgical procedures, only identify the procedure(s) and enter "See Appendix 5 for details".)

Procedure	Monitoring (methods, frequency and duration of monitoring through post-procedure recovery)	Person(s) responsible for monitoring	Method(s) for alleviating pain/ distress, during or after procedure (dose, route, duration of effect)

Tumor biopsy	While sedated, the dog's' behavior will be monitored continuously until the dog is ambulatory. Pulse and respiratory rate will be monitored every 5 minutes until the dog is ambulatory. Post procedure pain/discomfort will be monitored by the clinician while the dog is in the clinic and once discharged, then by the dog's owner.	[redacted]	Sedation with butorphanol (0.2-0.4 mg/kg IM or IV) and or midazolam (0.2 mg/kg IV) – duration of effect is approximately 45 minutes. Lidocaine at the site of the biopsy for pain (0.5-0.75 ml 2% Lidocaine, intradermal) – duration of effect is approximately 45 minutes. Post biopsy pain will be alleviated with analgesics for 3 days or longer if the clinician or owner perceives that the dog is experiencing continued discomfort. Analgesics may include either Carprofen (2.2 mg/kg, orally, twice daily, Deracoxib (3-4 mg/kg, orally, 1x daily), or Meloxicam (0.1-0.2 mg/kg, orally, 1x daily); and/or Tramadol (1-5 mg/kg, orally, every 8 hr)
IT-IC administration	While sedated, the dog's behavior will be monitored continuously until the dog is ambulatory. Pulse and respiratory rate will be monitored at least every 15 minutes. Post procedure pain/discomfort will be monitored by the clinician while the dog is in the clinic and once discharged, then by the dog's owner.	[redacted]	Sedation with butorphanol (0.2-0.4 mg/kg IM or IV) and or midazolam (0.2 mg/kg IV) – duration of effect is approximately 45 minutes. Lidocaine at the site of the IT-IC administration for pain (0.5-0.75 ml 2% Lidocaine, intradermal) – duration of effect is approximately 45 minutes. Post IT-IC administration pain will be alleviated with analgesics for 3 days or longer if the clinician or owner perceives that the dog is experiencing continued discomfort. Analgesics may include either Carprofen (2.2 mg/kg, orally, 2x daily, Deracoxib (3-4 mg/kg, orally, 1x daily), or Meloxicam (0.1-0.2 mg/kg, orally, 1x daily); and/or Tramadol (1-5 mg/kg, orally, every 8 hr)
Radiation therapy	Dogs will be closely monitored by the anesthesia technician (specifically heart rate, pulse ox, and respiratory rate) throughout anesthesia and at least every 15 minutes after until the dog is ambulatory.	[redacted]	Etomidate (1-2 mg/kg, IV) and/or Propofol (4-5 mg/kg IV) to induce anesthesia – duration of effect is approximately 30 minutes). Isoflurane (1.2-1.3%, inhalation) and/or Sevoflurane (2.4%, inhalation) for anesthesia – duration is approximately 30 minutes. Percentages may be adjusted depending on patient's clinical response to anesthesia under guidance of the clinician.

Anti-PD-1 antibody	Dogs will be monitored every 2 hours for 6 hours post administration by the clinician post administration (heart and respiratory rate, body temperature). For determination of adverse effects, dogs will be monitored by the veterinary oncologist at each clinic visit- including a complete physical examination and at the indicated timepoints in the study protocol, a complete blood count, serum biochemistry profile, and urinalysis.	[redacted] and/or the Oncology Clinical Trial Intern; and the pet dog's owner	If an adverse reaction is observed, the attending clinician will prescribe appropriate treatment and/or supportive care. After discharge from VMTH, owners will monitor their dogs for development of adverse effects including lethargy, vomiting, diarrhea, anorexia, respiratory distress, or any other changes in their dog's behavior.
Administration of IT-IC, RT, and/or Anti-PD-1 antibody may result in an adverse event(s)	Physical examination and patient history will be assessed at each clinic visit. A CBC, biochemistry profile, and urinalysis will be performed at the times indicated in the Treatment and Evaluation Schedule tables in Item C.2.c. above. Adverse events will be graded according to the 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 Dec; 14(4): 417-446. Dose-limiting toxicity (severe adverse event) will be a grade 3 in any adverse event category except for neutropenia, where a grade 4 toxicity is considered dose-limiting (severe).	[redacted] and/or the Oncology Clinical Trial Intern and the pet dog's owner.	If an adverse reaction is observed, the attending clinician will prescribe appropriate treatment and/or supportive care. The attending clinician may remove a dog from this study if it is determined that there are severe side-effects that are not able to be managed with supportive care – this may include severe diarrhea, vomiting, and/or hematologic (e.g., neutropenia) or biochemistry alterations. An owner may remove their dog from this study at any time for any reason. If a dose-limiting toxicity is observed, treatment may be delayed and/or dose-decreased or discontinued.

K. **Justification of Category E procedures.** Indicate which statement below applies, and provide the information requested.

► (**X**) This protocol does NOT include any Category E procedures

► ( ) This protocol INCLUDES Category E procedures.

Veterinary Care and Husbandry

L. **Veterinary Support**.

a. Identify the laboratory animal veterinarian who is responsible for ensuring that the animals on this protocol receive appropriate veterinary medical care.

VA
Name► [redacted]**, DVM, MS**
Institutional affiliation► **VMC**
email contact► [redacted]

UW SVM
Name► [redacted]**, DVM, DACLAM**
Institutional affiliation► **UW School of Veterinary Medicine**
email contact► [redacted]**; cell:** [redacted]

b. Veterinary consultation during the planning of this protocol.
Name of the laboratory animal veterinarian consulted► [redacted]**, DVM, DACLAM**
Date of Veterinary consultation (meeting date, or date of written comments received by PI)► **02/01/2018**

M. **Husbandry.** For the animal care staff, summarize the animal husbandry requirements on this protocol.

- The *Guide* states that social animals should generally be housed in stable pairs or groups.
- The ARF Housing and Enrichment SOPs are entered in Item Y
- Use Appendix 6 to justify the use of any special husbandry and to detail its effects on the animals.
- Use Appendix 9 to document any "departures" from the standards in the *Guide*.
- Consult the IACUC or Attending Veterinarian for help to determine whether any departures are involved.

a. **Caging needs.** ACORP Instructions provide guidance on describing the type of housing needed:

- Describe the housing to be accommodated by the housing sites for this protocol:

a. Type of housing*	**b**. Number of individuals per housing unit**	**c**. Is this housing consistent with the *Guide* and USDA regulations?	**d**. Est. maximum number of housing units needed at one time
☐ **Standard – See SOP in Item Y for Non-sterile rodent micro-isolator caging with filtered cage top**		☐ Yes ☐ No***	
☐ **For standard housing not described in the ARF SOP**: in Item Y, check that Appendix 6 will be completed and describe the standard housing in this location			
☐ ***Justification for single animal housing is provided in **Appendix 9** as a departure from the *Guide* standards (check Appendix 9 in Item Y and complete ACORP Appendix 9)			
☒ Single animal housing that is not considered a departure from the standards in the *Guide*. **Rationale**: **Patient-owned animals being treated in a clinical setting. Owner will be given UW Veterinary Care (UWVC) Patient Discharge Instructions. In the event a dog must stay overnight at UWVC, the dog will be housed in single animal housing.**			

b. **Enrichment.** Indicate whether "standard" exercise and environmental enrichment will be provided to the animals on this protocol, or whether any special supplements or restrictions will be required.

- ACORP Instructions provide information on enrichment requirements.
- Use Appendix 9 to document any enrichments requirements that represent "departures" from the standards

in the *Guide*.

e. Description of Enrichment*	f. Frequency
☐ **Standard ARF SOP– See entry in Item Y**	**n/a**
☐ **Enrichment detailed in alternate SOP - See approved SOP listed in Item Y**	
☐ **Non-standard enrichment - See description in Appendix 9**	
☒ **Standard – see below***	
***Patient-owned animals being treated in a clinical setting. Owner will be given UW Veterinary Care Patient Discharge Instructions.**	

c. **Customized routine husbandry**. Check the statements below that apply to this protocol.
Provide instructions to the animal care staff with regard to any customized routine husbandry needed.

► (**N/A**) **Genetically modified animals** are included on this ACORP.
Describe each group of genetically modified animals:
- Expected characteristic clinical signs or abnormal behavior related to genotype?
- Any customized routine husbandry required to address these?
- Genetic modifications that will be newly generated on or for this protocol?

► (**X**) **Additional monitoring** of animals will be required under this ACORP.
- Describe any special attention needed during routine husbandry to monitor for unexpected clinical signs or abnormal behavior that may require customized routine husbandry.

►Dogs in the proposed study will be monitored both by the dog's owner and clinicians at UW Veterinary Care for development of adverse reactions. Toxicity evaluations will be performed by the clinician at each clinic visit. This will include a physical examination and, at indicated times in the study protocol, this will also include a complete blood count, serum biochemistry profile, and urinalysis. If an adverse reaction is observed, the attending clinician will prescribe appropriate treatment and/or supportive care.

► (**N/A**) **Devices that extend chronically through the skin** WILL be implanted into some or all animals on this protocol.
Describe any customized routine husbandry to be provided by animal husbandry staff to minimize the chances of chronic infection where the device(s) penetrate the skin.

► (**N/A**) **Additional customized routine husbandry** by the animal husbandry staff will be required.
Describe the special husbandry needed and fill out Item V.

► (**X**) **No customized routine husbandry required** for animals on this ACORP.

N. **Housing Sites**. Document below <u>each location</u> where animals on this protocol may be housed.

► (**N/A**) Housing on **VA** property. Identify each location and indicate if the location is inside the VMU.

Building	Room number	Inside of VMU?	
		Yes	No
		( )	( )

► (**X**) Housing in **non-VA** facilities. Identify each location (UW) where animals on this protocol will be housed, and provide the information requested in the table.

Name of Non-VA Facility	Facility accredited by AALAC?	Building	Room

	Yes--enter status*	No**		
UW Veterinary Care Patient-owned animals being treated in a clinical setting. Owner will be given UW Veterinary Care Patient Discharge Instructions. In the event a dog must stay overnight at UWVC, the dog will be housed in single animal housing.	(**X**) **Full accreditation**	( )**	School of Veterinary Medicine (SVM)	Oncology ward

*See ACORP Instructions, for a list of AAALAC accreditation status options.

**For any facility listed above that is not accredited by AAALAC, attach documentation that a waiver has been granted by the CRADO.

Special Features

O. **Antibody Production.** Will any of animals on this protocol be used for the production of antibodies?

► ( ) Some or all of the animals on this protocol WILL be used in production and harvesting of antibodies.
*Check "Appendix 2" in Item Y, below, and complete and attach Appendix 2, "Antibody Production".

► (**X**) NO animals on this protocol will be used in the production and harvesting of antibodies.

P. **Biosafety.** Will any substances (other than those used in routine husbandry or veterinary care) be administered to the animals on this protocol?

► (**X**) This protocol INVOLVES administration of substances to the animals other than those used in routine husbandry and veterinary care.
*Check "Appendix 3" in Item Y, below, and complete and attach Appendix 3, "Biosafety".

► ( ) This protocol does NOT involve administration of any substances to the animals other than those used in routine husbandry and veterinary care.

Also answer the following:
► ( ) All compounds given to living animals will be pharmaceutical grade. --OR --
► (**X**) All compounds given to living animals will be pharmaceutical grade, except for the following:
(provide a list of compounds, justify the use of each one, and describe how it will be compounded):

► Clinical grade hu14.18-IL2, immunocytokine (IC) will be supplied by Apeiron Biologicals. The IC will be from the same batch (Batch Y12A972) which will be administered to human melanoma patients in the UWCCC protocol # UW16134 "Phase I/II Trial of Intratumoral Administration of Hu14.18-IL2, with Local Radiation, Nivolumab and Ipilimumab in Subjects with Advanced Melanoma". However, as an Investigational New Drug (IND) approval of UW16134 is still pending, this batch of hu14.18-IL2 cannot be imported into the US as clinical material. As such, the material will be declared for nonclinical use. Moreover, this batch of hu14.18-IL2 has been previously well tolerated when used at UWVC in tumor-bearing dogs at the doses proposed in this study (Protocol Title: "Phase I Dose-Finding Trial of IT-IC in Tumor-Bearing Dogs", IACUC approval number [redacted]**). Dr.** [redacted]**, is an established collaborator with Drs.** [redacted] **and** [redacted] **for the preclinical and clinical development of the IC.**

Q. **Locations of procedures.** Detail the location where each procedure is performed animals listed under this protocol. Record if the procedure will be performed at a VA or UW facility, list location(s) at the animal facility, then name each procedure to be performed, and if transport is required.

Facility VA/UW	Bldg/Room Number	Procedure	Surgical?		Requires transport through VA non-research areas? Policy #32 describes approved methods of animal transport	
			Yes	No	Yes – describe method of discreet transport	No
UW Veterinary Care (VMTH)	Oncology ward	Tumor biopsy, blood and urine collection, and administration of IT-IC and anti-PD-1 antibody.	**(Y)**	**( )**	**( )**	**(X)**
UW Veterinary Care (VMTH)	Radiation Therapy Suite	Anesthesia and radiation therapy	**( )**	**(N)**	**( )**	**(X)**
UW Veterinary Care (VMTH)	Diagnostic Imaging	Thoracic radiographs	**( )**	**(N)**	**( )**	**(X)**

Transporting? Add a concise statement as to the reason animals must leave the animal housing facility and enter the VA. ►**N/A**

R. **Body Fluid, Tissue, and Device Collection.** List body fluid, tissue, or device to be collected, and indicate the nature of the collection. If ante mortem collections are planned, complete the relevant Appendix and record completion in Item Y.

Body Fluid, Tissue, or Device to be Collected	Collected **AFTER** Euthanasia	Collected **BEFORE** Euthanasia (**Appendixes required**)		
		Blood Collection for Antibody Production (Appendix 2)	Collected as Part of Surgical Procedure (Appendix 5)	Other Collection from Live Animals (Appendix 4)
Urine	**(-)**	**(-)**	**(-)**	**(X)**
Tumor biopsy	**(-)**	**(-)**	**(X)**	**(-)**
Fine needle aspiration	**(-)**	**(-)**	**(-)**	**(X)**
Peripheral blood	**(-)**	**(-)**	**(-)**	**(X)**

S. **Surgery.** Does this protocol include any surgical procedure(s)?

► (**X**) Surgery WILL BE PERFORMED on some, or all, animals on this protocol.
Check "Appendix 5" in Item Y, below, and complete and attach Appendix 5, "Surgery".

► ( ) NO animals on this protocol will undergo surgery.

T. **Endpoint criteria.** Describe criteria to be used to determine when animals will be removed from the protocol or euthanatized to prevent suffering.

- Use Appendix 9 to document any "departures" from the *Guide* represented by these criteria.
- Consult the IACUC or the Attending Veterinarian to determine whether any departures are involved.
- Review (Body Condition Scoring: A Rapid and Accurate Method for Assessing Health Status in Mice. Mollie H. Ullman-Culleré and Charmaine J. Foltz. Laboratory Animal Science. Vol 49, No 3, June 1999)

► The study endpoint is the time the dog develops progressive disease as determined by physical examination and/or thoracic radiographs.

An owner may remove their dog from this study for any reason. The attending clinician may remove a dog from this study if it is determined that there are severe side-effects that are not able to be managed with supportive care – this may include severe diarrhea, vomiting, and/or neutropenia.

Physical examination and patient history will be assessed at each clinic visit. A CBC, biochemistry profile, and urinalysis will be performed at the times indicated in the Treatment and Evaluation Schedule tables in part 2c of this application. Severe adverse events will be classified as grade 3 according to the veterinary cooperative oncology group-common terminology criteria for adverse events (VCOG-CTCAE) in any adverse event category except for neutropenia, where a grade 4 toxicity is considered severe (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 Dec; 14(4): 417-446 – see table below criteria for adverse event grades.). For example, a grade 4 neutropenia would be an absolute neutrophil count of <500x10^3 cells/μL blood.

Euthanasia is not a study endpoint nor is it part of this study. Euthanasia is only performed at the request of a dog's owner.

Adverse Event (AE) Grades Grade refers to the severity of the AE. The VCOG-CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on this general guideline.	
Grade 1	Mild; asymptomatic or mild symptoms; clinical signs or diagnostic observations only; intervention not indicated.
Grade 2	Moderate; minimal, outpatient or non-invasive intervention indicated; moderate limitation of activities of daily living (ADL).
Grade 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; significantly limiting ADL.
Grade 4	Life-threatening consequences; urgent interventions indicated,
Grade 5	Death related to AE.

U. **Termination or removal from the protocol**.

- Use Appendix 9 to document any "departures" from the *Guide* represented by stated methods of disposition or euthanasia.
- Consult the IACUC or the Attending Veterinarian to determine whether any departures are involved.

Complete each of the following that applies:

► (**X**) Some or all animals will NOT be euthanatized on this protocol.
Describe the disposition of these animals.
►**Dogs will remain with their owner.**

► ( ) Some or all animals MAY be euthanatized as part of the planned studies.
Describe the exact method(s) of euthanasia to be used in the table below.

Method may be used on this protocol	Method of Euthanasia	AVMA Classification

		Acceptable	Conditionally Acceptable	Unacceptable
( )	**CO_2 from a compressed gas tank** Duration of exposure after apparent clinical death► **N/A** Method for verifying death► **N/A** Secondary physical method► **N/A**	( )	( )	( )
( )	**Anesthetic overdose** Agent► **N/A** Dose► **N/A** Route of administration► **N/A**	( )	( )	( )
( )	**Decapitation under anesthesia** Agent► **N/A** Dose► **N/A** Route of administration► **N/A**	( )	( )	( )
( )	**Exsanguination under anesthesia** Agent► **N/A** Dose► **N/A** Route of administration► **N/A**	( )	( )	( )
(**X**)	**Other (Describe) ► Although euthanasia is not an endpoint of this study, if a dog's owner requests euthanasia, the dog will be euthanized by barbiturate overdose. Agents used for euthanasia may include for example Beuthanasia or Fatal-Plus (1 mL/10 lbs., administered IV). Each ml of these agents contains 390 mg of pentobarbital sodium.** **Death will be confirmed by a lack of heartbeat and respiration.**	(**X**)	( )	( )

a. For each method designated as "Conditionally Acceptable" by the AVMA, describe how the conditions for acceptability will be met:
► **N/A**

b. For each method designated as "Unacceptable" by the AVMA, give the scientific reason(s) that justify this deviation from the AVMA Guidelines:
► **N/A**

c. **Identify all research personnel** who will perform euthanasia on animals on this protocol; describe their training and experience with the methods of euthanasia they are to use in the species indicated.
► **Either** [redacted] **or the Oncology Clinical Trial Intern will perform euthanasia if requested by the dog's owner. Both Dr.** [redacted] **and the Intern are licensed veterinarians experienced with performing euthanasia using this method in dogs.**

d. **Instructions for the animal care staff** in case an animal is found dead.
i. Describe disposition of the carcass, including any special safety instructions.
If disposition is to be handled according to a local SOP, enter "according to local SOP" and enter the specific SOP information into the table in Item Y.
► **If a dog is found dead, the dog's owner will decide the dog's disposition.**

Describe how the PI's staff should be contacted.
► (**X**) **Please contact a member(s) of the PI's staff immediately**.
Name► [redacted]

Contact Information► [redacted]; email: [redacted]

► ( ) There is no need to contact the PI's staff immediately.

Describe the routine notification procedures that will be followed, if procedures are described in a local SOP, enter "according to local SOP" and enter the specific SOP into Item Y.
►[redacted] **will be notified and he will notify the dog's owner. The owner will decide the disposition of the dog.**

V. **Special Procedures.** List each special procedure that is a part of this protocol (include special husbandry and other special procedures) and specify where the details of the procedure are documented.
- If any special procedure is detailed in a SOP, identify the SOP here, then enter the information in Item Y.
- If any special procedure is detailed in Appendix 6, check "Appendix 6" here and in Item Y, below.
- Use Appendix 9 to document any "departures" from the *Guide* represented by these procedures.
- See ACORP Instructions for examples.

Name of Procedure	Where the Details of the Procedure are Documented		
	SOP (title or ID number)*	Other Items in this ACORP-specify the Item letter(s)	Appendix 6
Radiation therapy treatment		Items:	(X)
IT-IC administration		Items:	(X)
Anti PD-1 antibody administration		Items:	(X)
Thoracic radiographs		Items:	(X)
Fine needle aspirate of draining lymph node		Items:	(X)
Blood collection		Items:	(X)
Urine collection		Items:	(X)

W. **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.

a. **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	Date of	Years	Potentially	Key words and/or search	Indicate which mandate each search addressed

database	search	covered by the search	painful or distressing procedures addressed	strategy used	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 "Anim al Use Alternative s" 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, anti-PD1	(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)	( )
PubMed	3/6/18	All years available	N/A	canine melanoma, anti-PD1, hu14.18-IL2	( )	( )	( )	(X)
PubMed	3/6/18	All years available	Cancer therapy adverse events	Adverse events and anti-PD1 treatment, adverse events and hu14.18-IL2 treatment, adverse events and radiation therapy			(X)	

Please use the **Animal Research Alternatives and Animal Care Guide** for literature searches to demonstrate the search for alternatives to using animals in research and ways to minimize painful procedures: http://researchguides.library.wisc.edu/animalalternatives

b. **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 anti-PD1" 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 anti-PD1), 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 hu14.18-IL2.

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.

c. **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. [redacted], biostatistician at the University of Wisconsin to determine the minimum number of animals to be used. Please see section C2b for details.

d. **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 UW 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 UW 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. [redacted], 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.

e. 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, anti-PD1, hu14.18-IL2 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.

X. **Other Regulatory Considerations**.

a. **Controlled drugs**.

i. Complete the table below for each drug that is used in animals on this protocol and that is classified as a controlled substance by the DEA. See ACORP Instructions for explanations about requested information.

Controlled substances	Storage		Personnel Authorized to Access	Location for Use		Procurement	
	Double-locked	Not Double-locked*		VA Property	Not on VA Property	VA Pharmacy	Non- VA
Butorphanol	(X)	( )	UW Veterinary Care pharmacist	( )	(X)	( )	(X)
Sodium Pentobarbital (e.g., Beuthanasia, Fatal-Plus)	(X)	( )	UW Veterinary Care pharmacist	( )	(X)	( )	(X)
Midazolam	(X)	( )*	UW Veterinary Care pharmacist	( )	(X)	( )	(X)
Tramadol	(X)	( )*	UW Veterinary Care pharmacist	( )	(X)	( )	(X)

*For any controlled substance that will NOT be stored under double lock, with limited access, describe how it will be stored, and explain why this is necessary. ► **N/A**

ii. Check each statement below that applies, to confirm that all controlled substances used on this protocol will be procured according to VA pharmacy policies:

► ( ) Some controlled substances will used on VA property, and all of these will be obtained through the local VA pharmacy.

► (**X**) Some controlled substances will not be obtained through the local VA pharmacy, but none of these will be used on VA property. See the ACORP Instructions, for further information.

b. **Human patient care equipment or procedural areas**. Does this protocol involve use of any human patient care equipment or procedural areas?

► ( ) Yes, some human patient care equipment or procedural area(s) will be used for the animal studies on this protocol. Check "Appendix 7" in Item Y, below, and complete and attach Appendix 7, "Use of Patient Procedural Areas for Animal Studies".

► (**X**) No human patient care equipment or procedural areas will be used for the animal studies on this protocol.

c. **Explosive agents**. Does this protocol involve use of any explosive agent?

► ( ) Yes, some explosive agent(s) will be used on this protocol. Check "Appendix 3" and "Appendix 8" in Item Y, below, and complete and attach Appendix 8, "Use of Explosive Agent(s) within the Animal Facility or in Animals", as well as Appendix 3, "Biosafety".

► (**X**) No explosive agent(s) will be used as part of this protocol.

Y. **Summary of Attachments.** To assist reviewers, summarize which of the following apply to this ACORP.

Appendices. Indicate which of the Appendices are required and have been completed and attached to this protocol. Do not check off or attach any appendices that are not applicable to this ACORP.

► (**X**) Appendix 1, "Additional Local Information" **Information on recruitment, inclusion criteria, blood collection from controls, and pretreatment evaluations are included in Appendix 1**

► (N/A) Appendix 2, "Antibody Production"

► (**X**) Appendix 3, "Biosafety"

► (**X**) Appendix 4, "Ante-mortem Specimen Collection"

► (**X**) Appendix 5, "Surgery"
► (**X**) Appendix 6, "Special Husbandry and Procedures"
► (N/A) Appendix 7, "Use of Patient Care Equipment or Areas for Animal Studies"
► (N/A) Appendix 8, "Use of Explosive Agent(s) within the VMU or in Animals"
► (N/A) Appendix 9, "Departures from "Must" and "Should" Standards in the *Guide*"
► (N/A) Appendix 10 "Breeding"
► (N/A) Appendix 11 "Overnight Housing in Labs"

Standard Operating Procedures (SOPs). List in the table below, each of the SOPs referred to in this protocol, providing the information requested for each one. The approved SOPs must be included when the approved ACORP and Appendices are submitted for Just-in-Time processing before release of VA funding support.

Item	SOP		Approval Date
	Title	ID	

Z. **Certifications.** Signatures are required here for any ACORP that is to be submitted to VA Central Office in support of an application for VA funding. Include the typed names and dated signatures as shown below for the Main Body of the ACORP and for each of the Appendices that apply to this protocol.
Do NOT include signatures for, or attach, any appendices that do NOT apply.

1. **Main Body of the ACORP.**

a. Certification by Principal Investigator(s):

Informed consent

I certify that, to the best of my knowledge, the information provided in this ACORP is complete and accurate, and the work will be performed as described here and approved by the IACUC. I understand that IACUC approval must be renewed at least annually, and that the IACUC must perform a complete *de novo* review of the protocol at least every three years, if work is to continue without interruption. I understand further that I am responsible for providing the information required by the IACUC for these annual and triennial reviews, allowing sufficient time for the IACUC to perform the reviews before the renewal dates, and that I may be required to complete a newer version of the ACORP that requests additional information, at the time of each triennial review.

Changes in protocol

I understand that further IACUC approval must be secured before any of the following may be implemented:

- Use of additional animal species, numbers of animals, or numbers of procedures performed on individual animals;
- Changing any procedure in any way that has the potential to increase the pain/distress category to which the animals should be assigned, or that might otherwise be considered a significant change from the approved protocol;
- Performing any additional procedures not already described in this ACORP;
- Use of any of these animals on other protocols, or by other investigators.

Changes in personnel on a protocol

I further certify that:

- No personnel will perform any animal procedures on this protocol until the IACUC has confirmed that they are adequately trained and qualified, enrolled in an acceptable Occupational Health and Safety Program, and meet all other criteria required by the IACUC.

- When new or additional personnel are to work with the animals on this protocol, I will provide this information to the IACUC for confirmation before they begin work;
- I will provide my after-hours contact information to the animal care staff for use in case of emergency.

Name of Principal Investigator	Signature	Date
[redacted], MD		

b. **Certification by IACUC Officials.**

We certify that:

- We, with the IACUC, have evaluated the care and use of animals described on this ACORP, in accordance with the provisions of the USDA Animal Welfare Act Regulations and Standards, PHS Policy, the *Guide for the Care and Use of Laboratory Animals*, and VA Policy;
- The IACUC has determined that the care and use of animals described in this ACORP is appropriate, and has therefore approved the protocol;
- The full text of any minority opinions is documented here as indicated below:
  - ► ( ) No minority opinions were submitted by any IACUC participant for inclusion.
  - ► ( ) Minority opinions submitted by IACUC participants are copied here ►
  - ► ( ) Minority opinions submitted by IACUC participants are attached on separate pages labeled "IACUC Minority Opinion" (indicate the number of pages►)

Name of Attending Veterinarian (VMC)	Signature	Date
[redacted], DVM		
Name of IACUC Chair	**Signature**	**Date**
[redacted], PhD		

2. **Appendix 2. Antibody Production.** Not applicable.

3. **Appendix 3. Biosafety.**

a. **Certification by PI(s) and IACUC Officials:**

We certify that:

- Before any animal experiments involving hazardous agents (identified in Appendix 3Item 10.a) are performed, SOPs designed to protect all research and animal facility staff as well as non-study animals will be developed and approved by the appropriate safety committee, VA or affiliated university, and by the IACUC;
- All personnel who might be exposed to hazardous agents (identified in Appendix 3Item 10.a) will be informed of possible risks and properly trained ahead of time to follow the SOPs to minimize the risks of exposure.

Name of Principal Investigator	Signature	Date
[redacted], MD		
Name of Institutional Veterinarian	**Signature**	**Date**

[redacted], DVM		
Name of IACUC Chair	**Signature**	**Date**
[redacted], PhD		

b. **Certification by Biosafety Official.** I certify that:

- Each agent to be administered to animals on this protocol has been properly identified in Item 1 of Appendix 3 as to whether it is "toxic", "infectious", "biological", or "contains recombinant nucleic acid";
- The use of each of the agents thus identified as "toxic", "infectious", or "biological", or "contains recombinant nucleic acid" is further documented as required in Items 4, 5, 6, and/or 8, as applicable, and in Appendix 3 Item 10.a;
- The use of each of these agents has been approved by the appropriate committee(s) or official(s), as shown in Item 10.a of Appendix 3.

Name of the Biosafety Officer	Signature	Date
[redacted], PhD		

c. **Certification by Radiation Safety Official.** I certify that:

- Each agent to be administered to animals on this protocol has been properly identified in Item 1 of Appendix 3 as to whether it is "radioactive";
- The use of each radioactive agent is further documented as required in Items 7 and 10.a of Appendix 3;
- The use of each radioactive agent has been approved by the appropriate committee(s), as shown in Item 10.a of Appendix 3.

Name of the Radiation Safety Officer	Signature	Date
[redacted], BS		

4. Appendix 4. Ante-mortem Specimen Collection. No signatures required.

5. Appendix 5. Surgery. Certification by the PI(s). I certify that:

- To the best of my knowledge, the information provided in Appendix 5 of this ACORP is complete and accurate;
- The surgical procedures will be performed and the post-operative care (including administration of post-operative analgesics) will be provided as described;
- The spaces where any survival surgical procedures will be performed (listed in Item 4 of Appendix 5) are suitable for sterile/aseptic surgery;
- The names and contact information for research personnel to notify or consult in case of emergencies will be provided to the VMU supervisor and veterinary staff;
- Post-operative medical records will be maintained and readily available for the veterinary staff and the IACUC to refer to, and will include the following:
  - Identification of each animal such that care for individual animals can be documented.
  - Daily postoperative medical records for each animal, that include documentation of daily evaluation of overall health and descriptions of any complications noted, treatments provided, and removal of devices such as sutures, staples, or wound clips;
  - Documentation of the administration of all medications and treatments given to the animals, including

those given to reduce pain or stress.
- Daily records covering at least the period defined as "post-operative" by local policy.
- The signature or initials of the person making each entry, live and in pen.

Name of Principal Investigator	Signature	Date
[redacted], MD		

6. **Appendix 6. Special Husbandry and Procedures.** No signatures required.
7. **Appendix 7. Use of Patient Care Equipment or Areas for Animal Studies. *N/A***
8. **Appendix 8. Use of Explosive Agent(s) within the Animal Facility or in Animals. *N/A***
9. **Appendix 9. Departures from "Must" and "Should" Standards in the *Guide*. No signatures required.**
10. **Appendix 10. Breeding. *N/A***
11. **Appendix 11. "Over Night Housing Appendix" *N/A***

ACORP Appendix 1
ADDITIONAL LOCAL INFORMATION

Recruitment of companion (pet) dogs

- Pet dogs with melanoma will be recruited for the study by the UWVC oncology service. The oncology service has extensive experience in recruiting animal patients for clinical trials. Having a well-staffed oncology service and a dedicated clinical trial intern allows for adequate adherence to the patient treatment and evaluation schedule.
- 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.
- The owner must provide written, informed consent via our standardized consent form prior to enrolling in the study. Both owners of dogs with spontaneous melanoma as well as healthy control dogs must provide such consent.
- The owners of dogs with spontaneous melanoma will be informed that while their dog may benefit from participating in this study, we do not know if that will occur. We do not expect to cure their dog's cancer in this study.
- Owners of dogs with spontaneous melanoma will be given special financial considerations as a remuneration to participate. These financial considerations are being offered to encourage participation in research to obtain general scientific knowledge that could potentially improve the care of humans and canines with melanoma in the future. Specifically, the cost of all study-related evaluations, all study-related interventions, all study-related follow-up, and all study-related side effects (up to $5,000 payment/dog for study-related side effects) will be covered by the study whether the dog completes all of the study or is not able to do so due to early withdrawal from the study or to disease progression. The amount of the financial considerations for a dog with melanoma is expected to vary depending on the amount of study-related research evaluations, study-related interventions, study-related follow-up, and whether any study-related side effects were experienced by the dog. If a dog experiences an adverse event related to the study, the study will pay for management of that event (up to $5,000). It is noted that this financial consideration is limited to covering the costs specific to participating in this study or the costs related to an adverse event related to the study. Any side effect from the study interventions or from the research is considered an adverse event. The intent of the study is to cover all research-related costs for this study. The $5,000 limit for payment of an adverse event related to the study was selected based on the extensive experience of the study team that this amount should cover any injuries the dog may have if there is a research-related injury. While unlikely, it is possible that medical costs for a research injury could exceed this limit. The owner should decline having their dog participate in this study if this plan for covering possible study-related side effects is not acceptable to them.
- Based on our experience with similar clinical trials using privately owned animals as subjects, the financial considerations as well as the opportunity to receive cutting-edge treatment for their pet is sufficient motivation for owners to participate and complete this study.
- Healthy pet dogs without melanoma presenting to UWVC for routine preventive care will be recruited by the staff for collection of a blood sample to serve as normal controls. Owners of healthy dogs providing a blood sample will be offered $50 toward the examination fee for their scheduled UWVC visit

Inclusion Criteria

- Pet dogs with a histologically confirmed malignant melanoma diagnosis that have a readily palpable and accessible tumor site of at least 2.0 cm allowing for IT-IC administration.
- Pet dogs without melanoma to provide blood samples as normal controls.
- The owner must provide written, informed consent prior to enrolling in the study.

Blood Collection for Healthy Pet Control Dogs without Melanoma

- These dogs will donate 12 ml blood for flow cytometry and TCR repertoire analyses. This total amount will not exceed 7.5% of a dog's blood volume collected within any 7-day period. These dogs will not receive any treatment.

Pretreatment Evaluation for Pet Dogs with Melanoma

- Complete physical examination.
- Collection of a total of 12 ml blood for flow cytometry and TCR repertoire analyses. Serum obtained from processing the blood sample will be cryopreserved separately for possible *in vitro* assays.
- Collection of an additional 5 ml blood for a complete blood count and biochemistry profile. This total amount of blood (12 + 5 ml) will not exceed 7.5% of a dog's blood volume collected within any 7-day period.
- Collection of urine for urinalysis. It is our intention to collect urine by free catch (walking the dog and catching urine in a bowl), however, if that is not possible, then urine will be collected by cystocentesis which involves mild physical restraint of the dog while a 22 gauge needle on a 10 ml syringe is inserted through the abdomen into the dog's bladder. This is a common procedure in UWVC. In some cases, if the bladder is not easily palpated, a portable ultrasound probe will be used to locate the bladder.
- Tumor biopsy.
- Fine needle aspirate of tumor draining lymph node to assess for metastasis.
- Thoracic radiographs to assess for lung metastasis.

ACORP APPENDIX 3
BIOSAFETY

See ACORP App. 3 Instructions for more detailed explanations of the information requested.

1. **Summary of <u>All</u> Materials Administered to Animals on this Protocol.** Complete the table below for <u>all</u> materials to be administered to any animal on this protocol, indicating the nature of the material by marking EVERY box that applies, and indicating the BSL number for any infectious agents:

Material (Identify specific agent, device, strain, construct, isotope)	**Source** (Identify vendor or colleague, or specify which animals on this protocol will serve as donors)	**Nature of Material**						
		Toxic Agent (Item 4)	Infectious Agent (Item 5) -- Enter the CDC Biosafety Level (BSL 1, 2, 3, or 4)	Biological Agent (Item 6)	Radioactive Agent (Item 7)	Contains Recombinant Nucleic Acid (Item 8)	Routine Pre- or Post-Procedural Drug	Euthanasia agent
Gas for anesthesia (isoflurane, sevoflurane)	UWVC preferred vendors	( )	( )BSL_	( )	( )	( )	(**X**)	( )
Propofol	UWVC preferred vendors	( )	( )BSL_	( )	( )	( )	(**X**)	( )
Carprofen	UWVC preferred vendors	( )	( )BSL_	( )	( )	( )	(**X**)	( )
Deracoxib	UWVC preferred vendors	( )	( )BSL_	( )	( )	( )	(**X**)	( )

Meloxicam	UWVC preferred vendors	( )	( )BSL_	( )	( )	( )	(**X**)	( )
Tramadol	UWVC preferred vendors	( )	( )BSL_	( )	( )	( )	(**X**)	( )
Beuthanasia	UWVC preferred vendors	( )	( )BSL_	( )	( )	( )	( )	(**X**)
Fatal-Plus	UWVC preferred vendors	( )	( )BSL_	( )	( )	( )	( )	(**X**)
Butorphanol	UWVC preferred vendors	( )	( )BSL_	( )	( )	( )	(**X**)	( )
Etomidate	UWVC preferred vendors	( )	( )BSL_	( )	( )	( )	(**X**)	( )
Lidocaine	UWVC preferred vendors	( )	( )BSL_	( )	( )	( )	(**X**)	( )
Midazolam	UWVC preferred vendors	( )	( )BSL_	( )	( )	( )	(**X**)	( )
IT-IC (hu14.18-IL2)	Apeiron Biologics	( )	( )BSL_	(**X**)	( )	( )	( )	( )
Caninized anti-PD-1 antibody	Currently under negotiation	( )	( )BSL_	(**X**)	( )	( )	( )	( )

2. **Summary of How Materials will be Administered.** Complete the table below for each of the materials shown in the table in Item 1 above:

Material* (Identify specific agent, device, strain, construct, isotope)	**Dose** (mg/kg, CFU, PFU, number of cells, mCi) and **Volume** (ml)	**Diluent* or Vehicle***	**Route** of admin	**Frequency or duration** of admin	**Reason** for Administration and **Expected Effects**	Location of **Further Details** in this ACORP (specify " Main Body" or " App #" , and identify the Item)	Administration Under **Anesthesia, sedation, or tranquilization** (Y/N)	Material is **FDA approved** or **pharmaceutical grade USP** (Y/N)
Gas for anesthesia (isoflurane, sevoflurane)	Average Dose: Isoflurane at 1.2-1.3%; Sevoflurane at 2.4% NOTE: Average percentages are adjusted depending on patient's clinical response to anesthesia under guidance of the clinician.	n/a	Inhalation	Approximately 30 minutes	Anesthesia	Main Body, Item J	Y	Y

Propofol	4-6 mg/kg	n/a	IV	Once during each radiation therapy	Anesthesia	Main Body, Item J	Y	Y
Carprofen	2.2 mg/kg	n/a	PO	2x/daily for 3 days	Analgesia, alleviation of pain	App #5, Item 7C	N	Y
Deracoxib	3-4 mg/kg	n/a	PO	1x/daily for 3 days	Analgesia, alleviation of pain	App #5, Item 7C	N	Y
Meloxicam	0.2 mg/kg	n/a	PO	1x on day 1	Analgesia, alleviation of pain	App #5, Item 7C	N	Y
Meloxicam	0.1 mg/kg	n/a	PO	1x/daily for days 2 and 3	Analgesia, alleviation of pain	App #5, Item 7C	N	Y
Tramadol	1-5 mg/kg	n/a	PO	2x/daily for 3 days	Analgesia, alleviation of pain	App #5, Item 7C	N	Y
Beuthanasia	1mL/10 lbs.	n/a	IV	Once, terminal	euthanasia	Main body, Item U	Y	Y
Fatal-Plus	1mL/10 lbs.	n/a	IV	Once, terminal	euthanasia	Main body, Item U	Y	Y
Butorphanol	0.2-0.4 mg/kg	n/a	IM or IV	Once prior to administration of IT-IC or prior to performance of tumor biopsy	Sedation	App #5, Item 5b	N	Y
Etomidate	1-2 mg/kg	n/a	IV	Once at the time of each radiation therapy treatment	Anesthesia	Main Body, Item J	N	Y
Lidocaine	0.5 – 0.75 ml 2% Lidocaine	n/a	Intra-dermal	Once at the time of each tumor biopsy	Analgesia, alleviation of pain	App #5, Item 5b	Y	Y
Midazolam	0.2 mg/kg	n/a	IV	Once at the time of each tumor biopsy	Sedation	App #5, Item 5b	N	Y
IT-IC (hu14.18-IL2)	2-12 mg/kg	n/a	Intra-tumoral	1/day for 3 days	Study Treatment	App #6, Special Procedure 6	Y	N
Anti-PD-1 antibody	10 mg/kg	n/a	IV	Q 21 days for 2 treatments	Study Treatment	App #6, Special Procedure 9	N	Y

- Material, diluent, or vehicle that is listed as "FDA Approved" or labeled "USP" is pharmaceutical grade.
- Check on-line for formulations that are FDA approved for administration to humans: (http://www.fda.gov/Drugs/InformationOnDrugs/ucm129662.htm) or animals: (http://www.fda.gov/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/UCM042847).

For each each material and each diluent or vehicle to be used that is <u>not</u> pharmaceutical grade, explain here why the use of a non-pharmaceutical grade formulation is necessary, and describe how it will be ensured that the material is suitable for use. (See ACORP App. 3 Instructions, for specifics about the level of detail required.)

► Clinical grade hu14.18-IL2, immunocytokine (IC) will be supplied by Apeiron Biologicals. The IC will be from the same batch (Batch Y12A972) which will be administered to human melanoma patients in the UWCCC protocol # UW16134 "Phase I/II Trial of Intratumoral Administration of Hu14.18-IL2, with Local Radiation, Nivolumab and Ipilimumab in Subjects with Advanced Melanoma". However, as an Investigational New Drug (IND) approval of UW16134 is still pending, this batch of hu14.18-IL2 cannot be imported into the US as clinical material. As such, the material will be declared for nonclinical use. Moreover, this batch of hu14.18-IL2 has been previously well tolerated when used at UWVC in tumor-bearing dogs at the doses proposed in this study (Protocol Title: "Phase I Dose-Finding Trial of IT-IC in Tumor-Bearing Dogs", IACUC approval number V005831). Dr. [redacted]**, is an established collaborator with Drs.** [redacted] **and** [redacted] **for the preclinical and clinical development of the IC.**

3. **Anesthesia, Sedation, or Tranquilization.** Complete 3.a. and 3.b. below:

   a. For each material with "Y" entered in the "Administered Under Anesthesia" column of the table in Item 2 above, describe the anesthesia, sedation, or tranquilization to be used, identifying the anesthetic, sedative, or chemical tranquilizer, and detailing the dose, volume, and route of administration (Make sure that these agents are also included in Item 1 of this appendix, as materials to be administered):
   ► Gas for anesthesia will be used after the dog is anesthetized with propofol (4-6 mg/kg, ~0.6 mL/kg, IV) and etomidate (1-2 mg/kg, 0.5-1mL/kg, IV).
   Propofol for anesthesia will be used after the dog is anesthetized with etomidate (1-2 mg/kg, 0.5-1mL/kg, IV).
   Lidocaine for analgesia will be administered after the dog is sedated with butorphanol (0.2-0.4 mg/kg, 0.04 mL/kg, IM or IV) and midazolam (0.2 mg/kg, 0.2 mL/kg, IV).
   IT-IC (hu14.18-IL2) will be administered after the dog is sedated with butorphanol (0.2-0.4 mg/kg, 0.04 mL/kg, IM or IV) and midazolam (0.2 mg/kg, 0.2 mL/kg, IV).
   Beuthanasia or Fatal-Plus will be administered after the dog is sedated with butorphanol (0.2-0.4 mg/kg, 0.04 mL/kg, IM or IV) and midazolam (0.2 mg/kg, 0.2 mL/kg, IV).

   b. For each material with "N" entered in the "Administered Under Anesthesia" column of the table in Item 2 above, explain why no anesthesia, sedation, or tranquilization is necessary, or can be provided, and describe any alternate methods of restraint that will be used.
   ► Carprofen, Deracoxib, Meloxicam, and Tramadol are administered orally by the clinician and by the dog's owner. There is no need for any type of restraint to accomplish administration of these medications. Butorphanol, Etomidate, Midazolam, and Anti-PD-1 antibody are administered with minimal restraint by gently holding the dog on and examination table.

4. **Toxic Agents.** Complete the table below for each of the materials listed as a "toxic agent" in the table in Item 1 above, checking the all of the properties that apply (see ACORP App. 3 Instructions, for details).

Name of Toxic Agent	a. Mutagen	b. Carcinogen	c. Teratogen	d. Select Agent?			e. Other – specify toxic properties
				Not a Select Agent	Select Agent Used in Sub-threshold Quantities	Select Agent that Requires Registration/Approval	
N/A	( )	( )	( )	( )	( )	( )*	( )►

*For each "select agent" that requires registration/approval (copy the lines below for each agent):
Name of agent ► **N/A**
Registered with CDC or USDA ► **N/A**

Registration Number ► **N/A**
Registration Date ► **N/A**
Expiration Date of Registration ► **N/A**
Name of official who granted approval on behalf of VACO► **N/A**
Date of approval► **N/A**

5. **Infectious Agents.** Complete the table below for each of the materials listed as an "infectious agent" in the table in Item 1 above (see ACORP App. 3 Instructions, for details).

Name and BSL Number of Infectious Agent	a. ABSL Number*	b. Drug Sensitivity Panel Available? (Describe)	c. Select Agent?		
			Not a Select Agent	Select Agent used in Sub-threshold quantities	Select Agent that Requires Registration/ Approval
N/A		(Yes/No)	( )	( )	( )**

*Complete the following for each agent for which the ABSL Number given is less than the BSL Number shown (copy the lines below for each agent):
Name of agent ► **N/A**
Justification for applying ABSL measures that are less protective than those recommended ► **N/A**

**For each "select agent" that requires registration/approval (copy the lines below for each agent):
Name of agent ► **N/A**
Registered with CDC or USDA ► **N/A**
Registration Number ► **N/A**
Registration Date ► **N/A**
Expiration Date of Registration ► **N/A**
Name of official who granted approval on behalf of VACO► **N/A**
Date of approval► **N/A**

6. **Biological Agents.** Complete the table below for each of the materials listed as a "biological agent" in the table in Item 1 above (see ACORP App. 3 Instructions, for details).

Name of Biological Agent	Screening for Infectious Agents
hu14.18-IL2 immunocytokine	Merck contracted for QC testing, including sterility screen, and bacterial endotoxin; Batch Y12A972 passed testing.
Anti-PD-1 antibody	To be determined when manufacturer/vendor identified.

7. **Radioactive Agents.** Complete the table below for each of the agents listed as a "radioactive agent" in the table in Item 1 above (see ACORP App. 3 Instructions, for details).

Name of Radioactive Agent (specify the isotope)	Authorized Individual	Approving Committee or Official
N/A		

8. **Agents Containing Recombinant Nucleic Acid.** For each of the materials checked in the table in Item 1, above, as "contains recombinant nucleic acid", indicate which of the conditions applies (see ACORP App. 3 Instructions, for details).

Name of Agent that Contains Recombinant Nucleic Acid	Subject to the *NIH Guidelines for Research Involving Recombinant DNA Molecules*	Exempt
N/A	( )	( )

9. **Potential for Pain or Distress**. Complete the table below for each of the agents listed in Item 1, above, that is expected to have potentially painful or distressing effects on the animals (see ACORP App. 3 Instructions, for details).

Name of Agent	Nature of Potential Pain/Distress	Measures to Alleviate Pain/Distress
Anti-PD-1 antibody	The caninized anti-PD-1 antibody has been evaluated in a small number of dogs without cancer and in a recently completed study of dogs with cancer (including melanoma). Side effects have been rarely observed, however, possible side effects may include allergic reaction, decrease in appetite, nausea, and diarrhea.	If an adverse reaction is observed, the attending oncology clinician will prescribe appropriate treatment and/or supportive care.
Hu14.18-IL2	Side-effects in some people receiving this IC (hu14.18-IL2) intravenously included development of an allergic reaction, short-term flu-like symptoms (fever, aches, lethargy), a transient drop in blood pressure right after treatment, and short-term changes in white and red blood cell counts. It is less likely that dogs will experience these side-effects as the IC will be administered directly into the tumor. We do not anticipate observing any dose-limiting side effects even at the highest planned dose of 12 mg/m^2/day in this study because: 1) the highest dose proposed is the same dose given IV to children with neuroblastoma (2 mg/m^2/day for 3 days); 2) intratumoral dosing is predicted to induce less IL2-related toxicity than IV dosing; and 3) canine IL2 receptors, while known to be sensitive to human IL2, are expected to be less sensitive to human IL2 than are human IL2 receptors. In the unlikely event that a dose-limiting toxicity is observed in 2 or more dogs at the first dose level (2.0 mg/m^2/injection), a new dose level (dose level -1) will be defined which is half that of the first (1.0 mg/m^2/injection). If, on the other hand, dose level 3 has been reached without determining the maximal tolerated dose (MTD), this dose will be defined as the maximally administered dose (MAD). In addition, several tumor-bearing dogs with various cancers have been treated at the doses proposed in this study and no dose-limiting toxicity has been observed.	If an adverse reaction is observed, the attending oncology clinician will prescribe appropriate treatment and/or supportive care.

10. **Protection of Animal Facility Staff from Hazardous Materials.** Complete Items 10.a and 10.b, below, for each of the agents listed in the table in Item 1, above, as “toxic”, “infectious”, “biological”, “radioactive”, or “contains recombinant nucleic acid” (detailed in Items 4 – 8). This item specifically addresses members of the animal facility staff; protection of the research staff from each of these agents must be addressed in Item G of the main body of the ACORP. See ACORP App.3 Instructions, for details.

a. Complete the table below.

Name of Hazardous Agent	Approving Committee or Official	Institution (VA or affiliate)	Names of Animal Facility Staff Members at Risk
hu14.18-IL2, immunocytokine	Biosafety	VA	[redacted] and/or the Oncology Clinical Trial Intern
caninized anti-PD-1 antibody	Biosafety	VA	[redacted] and/or the Oncology Clinical Trial Intern

b. Detail how the individuals listed in the table above (Item 10.a.) have been (or will be) informed of the possible risks of exposure, and have been (or will be) trained to avoid exposure to these agents.

► Dr. [redacted] is a boarded veterinary oncologist who has performed the procedures described in this study in dogs since 1984. He is an established investigator in canine clinical trials using numerous treatment modalities, including delivery of biologics to canines. Moreover, Dr. [redacted] has experience administering intratumoral hu14.18-IL2, as described herein, to several tumor-bearing dogs with various cancers. Dr. [redacted] also has experience delivering monoclonal antibodies to canines. As such, he is aware of the potential risks of exposure to such agents.

The Oncology Clinical Trial Intern will work closely with Dr. [redacted] and will be informed of the risks involved and provided with the SOPs and training needed to minimize their risks of exposure to the biologics used in this study.

11. **Signatures.** Provide applicable signatures on the signature pages (Item Z.3) of the ACORP main body.

ACORP Appendix 4
ANTEMORTEM SPECIMEN COLLECTION

See ACORP App. 4 Instructions for more detailed explanations of the information requested.

1. **Summary.** Complete the table below for each specimen to be collected from a live animal on this protocol (see ACORP App. 4 Instructions, for details).

Specimen Collected	Site and Method of Collection	Anesthesia (Yes/No)	Amount Collected Each Time	Volume Replacement (Yes/No/NA)	Total Number of Collections per Animal	Time Intervals Between Successive Collections
Blood	Accessible vein (jugular, saphenous, or cephalic)	No	Approximately 30 mL - the maximum will not exceed 7.5% of a dog's blood volume within any 7-day period	No	5 maximum	Varies – once for healthy dogs used for assay controls and for patient dogs, the shortest interval would be 14 days
Urine	Free catch; cystocentesis if free catch is not feasible	No	10 mL	No	3	The shortest interval would be 14 days.
Tumor	Tumor Punch Biopsy	Yes	Punch biopsies (6-8 mm) will be collected by sterile technique and bisected	No	4	The shortest interval would be 7 days.
Fine needle aspirate of draining lymph node	Lymph node needle aspirate	No	20 μL	No	1	n/a

2. **Use of Anesthetics, Tranquilizers, or Analgesics**.

a. For each specimen described in Item 1, above, as being collected WITHOUT anesthesia, complete Items 2.a(1) and 2.a(2), below:

(1) Explain why no measures will be taken to prevent pain (e.g., because of scientific requirements described here, or because the collection method involves no more than minor or momentary pain).

► **Collection of blood and fine needle aspirate are performed with no more than minor or momentary pain and are routinely performed with minimal restraint. Collection of urine is done by free catch and does not involve any pain or restraint; if necessary cytocentesis may be used to collect urine and does not involve pain, and requires only mild restraint.**

(2) Completely describe any method of physical restraint that may be used.

►**Minimal restraint by gently holding the dog while a procedure is performed**.

b. For each specimen described in Item 1, above, as being collected WITH anesthesia, complete the following table:

Anesthetic, tranquilizer, or analgesic agent	Dose (mg/kg) and volume (ml)	Route of administration	Frequency of administration
N/A			

3. **Volume Replacement for Fluid Collections.**

   a. For each fluid specimen described in Item 1, above, for which NO volume replacement will be provided, explain why not.

   ► The amount of fluid collected (blood, urine, and lymph node aspirate) does not require volume replacement.

   b. For each fluid specimen described in Item 1, above, for which volume replacement WILL be provided, describe the replacement fluids that will be administered (including their composition, volume, and route of administration).

   ► N/A

4. **Monitoring the animals.** Detail how the animals will be monitored after collection of specimens to ensure that they recover appropriately (see ACORP App. 4 Instructions, for details).

► Monitoring is not necessary following urine collection. Dogs will be monitored by the oncology clinician immediately for bruising following blood collection. The tumor biopsy and lymph node aspirate sites will be monitored by the oncology clinician when the dog is in the clinic and by the dog's owner when the dog is discharged for signs of bleeding or infection.

ACORP Appendix 5
SURGERY

See ACORP App. 5 Instructions for more detailed explanations of the information requested.

1. **Surgery Classification.** Describe each surgery included in this protocol, and indicate how it is classified (terminal, minor survival, major survival, one of multiple survival). ACORP App. 5 Instructions, for details.

Surgery		Terminal	Survival		
#	Description		Minor	Major	One of Multiple*
1	**Tumor biopsy**	( )	()	( )	(**X**)*

*If survival surgery (including major surgeries and any minor surgeries that may induce substantial post-procedural pain or impairment) will be performed as part of this protocol in addition to any other such surgery (on this or another protocol) on the same individual animal, complete items 1.a and 1.b, below:

a. Provide a complete scientific justification for performing the multiple survival surgeries on an individual animal:

► **Sequential tumor biopsies are needed to assess the dog's immunological response to treatment.**

b. Give the interval(s) between successive surgeries, and the rationale for choosing the interval(s):

► **Tumor biopsies will be performed prior to and on study day 10, 17, and 24. The rationale for choosing these days are that they are within the expected window of observable immune response.**

2. **Description of Surgeries.** Describe each surgery listed in Item 1, providing enough detail to make it clear what the effects on the animal will be. (Pre-operative preparation, anesthesia, and post-operative recovery will be covered in items 5, 6, and 7, below.)

Surgery 1 ► **The area to be biopsied will be clipped and aseptically prepped with 2% chlorhexidine scrub and 70% isopropanol. A stab incision will be made with a #11 or #15 scalpel blade, and a biopsy obtained using the punch device. The skin edges will be apposed with a simple interrupted or cruciate suture using 3-0 nylon suture material. Tumor biopsies will take approximately 5 minutes. Sutures will be removed at the time of the next biopsy, or after 7 days.**

3. **Personnel.** List each individual who will be involved in any of the surgeries described in Item 1.

Name	Surgery #(s)	Role in Surgery			
		Surgeon	Assistant	Manage Anesthesia	Other (describe)
[redacted]	1	(**X**)	( )	( )	( )
Oncology Clinical Trial Intern	1	(**X**)	(**X**)	( )	( )

4. **Location of surgery.** List for each location where surgeries described in Item 1 will be performed.

Building	Room Number	Surgery #(s)	Type of Space		
			Dedicated Surgical Facility	Other Dedicated Surgical Space	Other Space not Dedicated to Surgery
UW Veterinary Care	Oncology ward	1	( )	( )*	(**X**)*

*For each space that is not in a dedicated surgical facility, provide the justification for using this space for surgery on this protocol
► **Tumor biopsies are routinely done within the UW Veterinary Care oncology ward.**

5. **Pre-operative protocol.**

a. **Pre-operative procedures.** List each pre-operative procedure that will be performed to prepare the animal(s) for surgeries described in Item 1.

Surgery #(s)	Fast (Specify Duration)	Withhold Water (Specify Duration)	Place Intravenous Catheter(s) (Specify Site(s))	Other – Describe
1	(**X**) – 12 hours	( ) --	( ) --	( ) --

b. **Pre-operative medications.** Describe agent(s) for induction of anesthesia, as well as any other pre-treatments that will be administered prior to preparation of the surgical site on the animal.

Agent	Surgery #(s)	Dose (mg/kg) & volume (ml)	Route of administration	Frequency of administration (e.g., times/day)	Pre-operative period of treatment (e.g., immediate, or # of days)
Butorphanol	1	0.2-0.4 mg/kg, 0.04 mL/kg	IM or IV	Once per biopsy	immediate
Midazolam	1	0.2 mg/kg, 0.2 mL/kg	IV	Once per biopsy	immediate
Lidocaine	1	2% Lidocaine, 0.5-0.75 mL	intradermal	Once per biopsy	immediate

c. **Pre-operative preparation of the surgical site.** For each surgery, identify each surgical site on the animals, and describe how it will be prepared prior to surgery.

Surgery 1 ► **The primary tumor will be biopsied. The area to be biopsied will be clipped and aseptically prepped with 2% chlorhexidine scrub and 70% isopropanol.**

6. **Intra-operative management. N/A**

a. **Intra-operative medications.** List each agent that will be administered to the animal during surgery.

Agent	Paralytic*	Surgery #(s)	Dose (mg/kg) & volume (ml)	Route of administration	Frequency of dosing
N/A	( )*				

* For each agent shown above as a paralytic, explain why its use is necessary, and describe how the animals will be monitored to ensure that the depth of anesthesia is sufficient to prevent pain. ► **N/A**

b. **Intra-operative physical support.** For each surgery, describe any physical support that will be provided for the animals during surgery (e.g., warming, cushioning, etc.). ► **N/A**

c. **Intra-operative monitoring.** Describe the methods that will be used to monitor and respond to changes in the state of anesthesia and the general well-being of the animal during surgery. Include how frequently intra-operative monitoring will occur.

► **The dog's behavior will be monitored continuously while sedated until the dog is ambulatory. Heart and respiratory rate will be monitored every 5 minutes until the dog is ambulatory, this is typically between 5-30 minutes following the conclusion of sedation.**

7. **Survival surgery considerations.** For each survival surgical procedure indicated in Item 1 and described in Item 2, complete Items 7.a. – 7.g.

a. Complete the table below for each survival surgery listed in Item 1, above.

Surgery #	Survival Period	Measures for Maintaining Sterility							
		Sterile Instruments	Surgical Cap	Sterile Gloves	Surgical Scrub	Sterile Drapes	Sterile Gown	Face Mask	Other*
1	Until natural death or owner-requested euthanasia.	(**X**)	( )	(**X**)	(**X**)	(**X**)	( )	( )	( )*

* Describe any "other" measures to be taken to maintain sterility during surgery.
► **N/A**

b. **Immediate post-operative support** provided to the animals after each surgery.

Surgery 1 ► **Dogs will be monitored until the patient is awake and ambulatory. Until the dog is awake, heart rate and respiratory rate will be monitored every 5 minutes.**

c. **Post-operative analgesia**. Complete the table below for each surgery listed in item 1, above.

Surgery #	Agent*	Dose (mg/kg) & Volume (ml)	Route of Administration	Frequency of Dosing (e.g., times/day)	Period of treatment (e.g. days)
1	**Carprofen**	2.2 mg/kg, pill	Oral	Twice daily	3 days
1	**Deracoxib**	3-4 mg/kg	Oral	Once daily	3 days
1	**Meloxicam**	0.2 mg/kg day 1, then 0.1 mg/kg days 2 and 3, volume based on a 30 kg dog = 4 mL on day one and 2mL on days 2 and 3	Oral	Once daily	3 days
1	**Tramadol**	1-5 mg/kg, pill	Oral	Every 8 hours	3 days
Analgesic prescribed will be determined by clinician; all four medications will not be prescribed.					

*For each surgery for which NO post-operative analgesic will be provided, enter "none" in the "Agent" column, and explain here why this is justified:
► **N/A**

d. **Other post-operative medication** descriptions, what will be administered as part post-operative care.

Surgery #	Medication	Dose (mg/kg) & Volume (ml)	Route of Administration	Frequency of dosing (e.g. times/day)	Period of treatment (e.g. days)
N/A					

e. **Post-operative monitoring**. <u>After-hours contact information for the personnel listed must be provided to the veterinary staff for use in case of an emergency.</u>

1. **Immediate** post-operative monitoring Include what signs are monitored.

Surgery #	Methods Of Monitoring	Frequency of Monitoring	Duration at this Frequency	Name(s) of Responsible Individual(s)
1	Pain, bleeding, pulse, respiration	Every 5 minutes	Until the dog is ambulatory. The dog is expected to be ambulatory between 5 and 30 minutes after completion of the biopsy.	[redacted] and/or the Oncology Clinical Trial Intern

2. **Prolonged** post-operative monitoring. Include what signs are monitored.

Surgery #	Methods Of Monitoring	Frequency of Monitoring	Duration at this Frequency	Name(s) of Responsible Individual(s)
1	Pain, bleeding, infection	Daily	One week	Dog's owner

f. **Consequences and complications** that may occur in the post-operative period.

1. **Common or expected post-operative consequences** or complications that may arise **and what will be done to address them.**

Surgery 1 ► **Dogs will be monitored in the hospital or by the dog's owners to make sure the dog is comfortable and not licking or chewing at the biopsy site. Owners will be instructed to contact UW Veterinary Care if their dog appears uncomfortable or if the biopsy site shows swelling, discharge, or pain when touched. If the dog is licking or chewing at the biopsy site, an Elizabethan collar will be placed on the dog until the biopsy site begins to heal (approximately 3-4 days). The tumor biopsy may cause mild discomfort for 2 to 3 days. Dogs will routinely receive analgesia for 72 hours following the procedure – longer if the clinician or the owner perceive the dog is experiencing continued discomfort. Analgesic agents will include one of the following: Carprofen; Deracoxib; or Meloxicam, and/or; Tramadol.**

2. **Criteria for euthanasia** related specifically to post-operative complications:

Surgery 1 ► **N/A**

3. **Emergency medical situation** interventions: if an emergency arises and none of the research personnel on the ACORP can be reached, identify any drugs or classes of drugs that should be avoided because of the scientific requirements of the project. (If the condition of the animal requires one of these drugs, the animal will be euthanatized instead.)

► **None**

8. **Maintenance of post-surgical medical records**. Complete the table below for each surgery, specifying where the records will be held, and identifying at least one individual who will be assigned to maintain accurate, daily, written post-surgical medical records. Indicate whether the named individuals are research personnel involved in this project, or members of the veterinary staff.

Surgery #	Location of Records	Name(s) of Individual(s) Responsible for Maintaining Written Records	Research Personnel	Veterinary Staff
1	UW Veterinary Care	[redacted] and/or the Oncology Clinical Trial Intern	( )	(**X**)

Certification. The PI must sign the certification statement in Item Z.5 of the main body of the ACORP.

ACORP APPENDIX 6
SPECIAL HUSBANDRY AND PROCEDURES

See ACORP App. 6 Instructions, for more detailed explanations of the information requested.

1. **Description of Procedures.** Complete the table below for each procedure listed in Item V of the main body of the ACORP that is not detailed in a SOP or in another item or Appendix of the ACORP. For each special procedure, check all features that apply.

Special Procedure		Features							
#	Brief Description	Husbandry	Restraint	Noxious Stimuli	Exercise	Behavioral Conditioning	rradiation	maging	Other**
1	**Patient husbandry**	(**X**)	( )	( )	( )	( )	( )	( )	( )
2	**Radiation therapy treatment**	( )	( )	( )	( )	( )	(**X**)	( )	( )
3	**Fine needle aspirate of lymph node**	( )	( )	( )	( )	( )	( )	( )	(**X**)
4	**Thoracic radiographs**	( )	( )	( )	( )	( )	( )	(**X**)	( )
5	**IT-IC administration**	( )	( )	( )	( )	( )	( )	( )	(**X**)
6	**Blood collection**	( )	( )	( )	( )	( )	( )	( )	(**X**)
7	**Urine collection**	( )	( )	( )	( )	( )	( )	( )	(**X**)
8	**Anti-PD-1 antibody administration**	( )	( )	( )	( )	( )	( )	( )	(**X**)

*Husbandry refers to all aspects of care related to the maintenance of the animals, including (but not limited to) provision of an appropriate diet, access to water, control of environmental conditions, and the selection of primary and secondary enclosures.

Describe any "Other" features that are involved. ► **N/A, see below:

a. Provide a complete description of each special procedure listed in Item 1 above, including the duration of the procedure, how frequently it will be repeated in any one animal, and any effects it is expected to have on the animal:

Special Procedure **1** ► **Patient husbandry**: Dogs will reside with their owner. If a dog does stay overnight in the clinic, the dog will be housed in the oncology ward of UW Veterinary Care and will be cared for by the attending oncology clinician and veterinary technicians.

Special Procedure **2** ► **Radiation therapy treatment**: Once an IT-IC dose is determined in Aim 1a, radiation therapy will be delivered to the primary tumor (the same site that IT-IC will be administered after radiation therapy is completed). Dogs in this part of the study (Aim 1b) will be randomized to receive radiation therapy as either a single 8 Gy fraction or in three 8 Gy fractions (given every other day for a total of 3). Based on the findings of Aim 1b, dogs in Aim 2 will receive either one or three 8 Gy fractions. Dogs will be under general anesthesia for approximately 30 minutes while the radiation therapy treatment is delivered. It is expected that the size of the primary tumor will decrease. Dogs receiving a single 8 Gy fraction will be treated on study day -6 and dogs receiving three 8 Gy fractions will be treated on study days -10, -8, and -6.

Special Procedure **3** ► **Fine needle aspirate of lymph node**: A fine needle aspirate of the tumor draining lymph node will be performed once prior to treatment to assess presence of melanoma cells. For this procedure, a 3 mL syringe with a 21 gauge needle is used. The needle is inserted into the lymph node

and the syringe barrel is pulled back to aspirate lymph node cells. This procedure takes approximately 30 seconds. This is a routine procedure and it is expected that it will not adversely affect the dog.

Special Procedure **4 ► Thoracic radiographs**: Dogs will be physically restrained for a few minutes for performance of thoracic radiographs. We do not anticipate any physical discomfort during these periods of restraint. These are short procedures and it would be less stressful ordinarily to forego sedation. However, if the dog is considered by the clinician or technician performing the procedure to be struggling too much to adequately perform the procedure, then sedation will be utilized. If sedation is necessary to perform these procedures, dogs will be given butorphanol as described previously. Thoracic radiographs will be performed prior to treatment and repeated on study day 30. This is a routine procedure and it is expected that it will not adversely affect the dog.

Special Procedure **5 ► IT-IC Administration**: Dogs will be sedated for IT-IC administration (butorphanol and midazolam). A maximum volume of 1 mL will be injected into the primary tumor. Injection will take approximately 15 seconds. IT-IC will be administered on 3 consecutive days (study days 1, 2, and 3). Toxicity evaluations will include a physical examination performed at each clinic visit and a complete blood count, biochemistry profile, and urinalysis performed on study days 10 and 24. Based on our previous experience with dogs receiving IT-IC, it is expected that administration will not adversely affect the dog.

Special Procedure **6 ► Blood collection**: Either a jugular, saphenous, or cephalic vein will be used for collection of blood samples. Blood samples (total of 5 mL) will be collected for complete blood counts and biochemistry profiles prior to treatment, and on study days 10 and 24. Blood will also be collected for *in vitro* assays (approximately 25 mL) prior to treatment and on study days 10, 30, and 60. Blood collection takes approximately 2 minutes. The amount of blood collected will not exceed 7.5% of the dog's blood volume within any 7-day period. This is a routine procedure and it is expected that it will not adversely affect the dog.

Special Procedure **7 ► Urine collection**: Urine will be collected by free catch prior to treatment and on study days 10 and 24. This is a routine procedure and it is expected that it will not adversely affect the dog. However, if a free catch is not possible, then urine will be collected by cystocentesis which involves mild physical restraint of the dog while a 22 gauge needle on a 10 ml syringe is inserted through the abdomen into the dog's bladder. This is a common procedure in UWVC. In some cases, if the bladder is not easily palpated, a portable ultrasound probe will be used to locate the bladder.

Special Procedure **8 ► Anti-PD-1 antibody administration**: Dogs in Aim 2 will receive anti-PD-1 antibody on study days -6 and 17. Anti-PD-1 antibody is administered intravenously over 30 minutes through a catheter in the cephalic of saphenous vein. For 6 hours post each antibody administration, monitoring will include body temperature, pulse, and respiratory rate every 2 hours after treatment. If an adverse reaction is observed, the attending clinician will prescribe appropriate treatment and/or supportive care. Toxicity evaluations will include a physical examination performed at each clinic visit and a complete blood count, biochemistry profile, and urinalysis performed on study days 10 and 24. Based on our previous experience with dogs receiving anti-PD-1 antibody, we do not expect any adverse effects.

b. <u>Explain why each of these special procedures is necessary</u>:

Special Procedure **1 ► Patient husbandry**: clients will provide husbandry and supportive care at home.

Special Procedure **2 ► Radiation therapy treatment**: The primary goal of this study is to determine whether IT-IC in combination with local radiation therapy and immune checkpoint blockade (anti-PD-1 antibody) is safe and has antitumor activity in canine melanoma. The study will also determine which treatment results in better immune activation.

Special Procedure **3** ► **Fine needle aspirate of lymph node**: This will be performed prior to treatment to determine if the lymph node draining the primary tumor is metastatic.

Special Procedure **4** ► **Thoracic radiographs**: These will be performed to assess presence of pulmonary metastasis.

Special Procedure **5** ► **IT-IC Administration**: The primary goal of this study is to determine whether IT-IC in combination with local radiation therapy and immune checkpoint blockade (anti-PD-1 antibody) is safe and has antitumor activity in canine melanoma. The study will also determine which treatment results in better immune activation.

Special Procedure **6** ► **Blood collection**: Blood will be collected for a complete blood count and chemistry profile to assess treatment toxicity. Blood will also be collected for *in vitro* assays to assess immune response to treatment.

Special Procedure **7** ► **Urine collection**: Urine will be collected for urinalysis to assess treatment toxicity.

Special Procedure **8** ► **Anti-PD-1 antibody administration**: The primary goal of this study is to determine whether IT-IC in combination with local radiation therapy and immune checkpoint blockade (anti-PD-1 antibody) is safe and has antitumor activity in canine melanoma. The study will also determine which treatment results in better immune activation.

2. **Personnel.** Complete the table below for each special procedure listed in Item 1, above. Identify the individual(s) who will be responsible for carrying out the procedures, and those who will be responsible for monitoring the condition of the animals during and after the procedures. After-hours contact information for the personnel listed must be provided to the veterinary staff for use in case of an emergency.

Procedure Number	Responsible Individual(s)	
	Carrying Out Procedure	Monitoring the Animals
1	Dog's owner	Dog's owner
2	[redacted]	[redacted]
3	[redacted] and the Oncology Clinical Trial Intern	[redacted] and the Oncology Clinical Trial Intern
4	Diagnostic imaging staff of UW Veterinary Care	Diagnostic imaging staff of UW Veterinary Care
5	[redacted] and the Oncology Clinical Trial Intern	[redacted] and the Oncology Clinical Trial Intern
6	[redacted] and the Oncology Clinical Trial Intern	[redacted] and the Oncology Clinical Trial Intern
7	[redacted] and the Oncology Clinical Trial Intern	[redacted] and the Oncology Clinical Trial Intern
8	[redacted] and the Oncology Clinical Trial Intern	[redacted] and the Oncology Clinical Trial Intern

3. **Potential Pain or Distress.** Complete the table below for each special procedure identified in Item 1, above, indicating for each procedure, whether potential pain and/or distress is expected, and, if so, describing the potential pain and/or distress and indicating whether any measures are to be taken to prevent or alleviate it.

Procedure Number	Expected Potential Pain and/or Distress			
	No	Yes		
		Description	To Be Relieved	Not to Be Relieved
1	(**X**)		( )[a]	( )[b]
2	(**X**)		( )[a]	( )[b]
3	(**X**)		( )[a]	( )[b]

4	(**X**)		( )[a]	( )[b]
5	( )	**Potential pain with IT-IC**	(**X**)[a]	( )[b]
6	(**X**)		( )[a]	( )[b]
7	(**X**)		( )[a]	( )[a]
8	( )	Based on our previous experience with dogs receiving anti-PD-1 antibody, we do not expect any adverse effects. However, it is possible that a dog may experience an allergic reaction, lethargy, nausea, vomiting, fever, diarrhea, or respiratory distress.	(**X**)[a]	( )[a]

a. For each procedure for which potential pain and/or distress is expected, but <u>WILL be prevented or alleviated</u> by administration of the analgesic(s) or stress-relieving agents, complete the table below:

Procedure Number	Agent	Dose (mg/kg) & vol (ml)	Route of admin	Freq of admin (times/day)	Duration of admin (days post-procedure)
1	**N/A**				
2	**N/A**				
3	**N/A**				
4	**N/A**				
5	Butorphanol	0.2-0.4 mg/kg, 0.04 mL/kg	IM or IV	Once prior to tumor biopsy	Once
	Midazolam	0.2 mg/kg, 0.2 mL/kg	IV	Once prior to tumor biopsy	Once
6	**N/A**				
7	**N/A**				
8	If an adverse reaction is observed, the attending clinician will prescribe appropriate treatment and/or supportive care. The medication administered will depend on the symptoms.				

Describe any non-pharmacological measures to be taken to address the potential pain and/or distress:

Special Procedure 1 ► **N/A**
Special Procedure 2 ► **N/A**
Special Procedure 3 ► **N/A**
Special Procedure 4 ► **N/A**
Special Procedure 5 ► **N/A**
Special Procedure 6 ► **N/A**
Special Procedure 7 ► **N/A**
Special Procedure 8 ► **N/A**

b. For each procedure for which potential pain and/or distress is expected and <u>will NOT be prevented or alleviated</u>, provide the scientific justification for this:

Special Procedure 1 ► **N/A**
Special Procedure 2 ► **N/A**
Special Procedure 3 ► **N/A**
Special Procedure 4 ► **N/A**
Special Procedure 5 ► **N/A**
Special Procedure 6 ► **N/A**
Special Procedure 7 ► **N/A**
Special Procedure 8 ► **N/A**

4. **Monitoring.** Describe how the condition of the animals will be monitored during and after each of the special procedures, and list the criteria that will be used to determine when individual animals will be removed from groups undergoing these procedures, because of pain or distress (see ACORP App. 6 Instructions, for details):

Procedure Number	Monitoring Methods	Endpoint Criteria
1	Dog's owner will monitor for signs of diarrhea, vomiting, lethargy, inappetance, and behavior changes.	An owner may remove their dog from this study for any reason. The attending clinician may remove a dog from this study if it is determined that there are severe side-effects that are not able to be managed with supportive care – this may include severe diarrhea, vomiting, and/or neutropenia. Severe would be classified as grade 3 according to the veterinary cooperative oncology group-common terminology criteria for adverse events (VCOG-CTCAE) in any adverse event category except for neutropenia, where a grade 4 toxicity is considered severe (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 Dec; 14(4): 417-446).
2	The radiation oncology staff will monitor the dog during and immediately following radiation therapy.	
3	The oncology clinician will monitor the dog for bleeding at the aspiration site.	
4	Dogs will be observed by the diagnostic radiation staff during this procedure.	
5	Dogs will be monitored for potential toxicity. Toxicity evaluations will include a physical examination performed at each clinic visit and a complete blood count, biochemistry profile, and urinalysis performed on study days 10 and 24.	
6	Dogs will be monitored by the oncology clinician immediately for bruising following blood collection	
7	Monitoring is not necessary following urine collection.	
8	For 6 hours post each antibody administration, monitoring will include body temperature, pulse, and respiratory rate every 2 hours after treatment. Dogs will be monitored for potential toxicity. Toxicity evaluations will include a physical examination performed at each clinic visit and a complete blood count, biochemistry profile, and urinalysis performed on study days 10 and 24.	

Secondary Just-In-Time ACORP Review

PI	STATION	CYCLE	APPLICATION TITLE
[redacted]	Madison, WI-607	MERIT/ Fall 2017	Administration of intratumoral immunocytokine to activate immune rejection of spontaneous canine melanoma

	SCORE	DESCRIPTION	ACTION NEEDED BY IACUC
○	0	No concerns noted. Any comments provided are for information only.	*None.* No further correspondence with the CVMO is needed; the ACORP(s) is(are) cleared and represent(s) no bar to funding the application.
●	1	Some concerns noted.	*The IACUC must review the **level 1** 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).* No further correspondence with the CVMO is needed; the ACORP(s) is(are) cleared and represent(s) no bar to funding the application.
○	2	Concerns are noted that must be addressed by the local IACUC and PI before funding can occur, but work described in the ACORP(s) may continue.	*A response to each of the **level 2** concerns noted below must be reviewed and cleared by the CVMO* before funding can be released. Upload the following at https://vaww.gateway.research.va.gov: (1) a memo addressing the concerns, dated and signed by the PI, veterinarian, and IACUC Chair; and (2) (a) revised ACORP(s) approved by the IACUC. *The IACUC must review each of the **level 1** concerns listed 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).*
○	3	Significant concerns are noted that must be addressed by the local IACUC and PI before funding can occur, and work described in the ACORP(s) listed below must cease immediately.	*A response to each of the **level 3** concerns listed below must be reviewed and cleared by the CVMO* before work can resume and funding can be released. (If unusual circumstances dictate that work should continue despite concerns, notify the CVMO immediately.) *A response to each of the **level 2** concerns noted below must be reviewed and cleared by the CVMO* before funding can be released. For **level 2 and 3** concerns, upload the following at https://vaww.gateway.research.va.gov : (1) a memo addressing the concerns, signed by the PI, veterinarian, and IACUC Chair; and (2) (a) revised ACORP(s) approved by the IACUC. *The IACUC must review each of the **level 1** concerns listed 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*

(cont.)

			into the ACORP(s).

The ACORP for Dr. [redacted] has received an overall score of 1, which means that it is cleared and represents no bar to funding the application, although some concerns were raised, as shown below.

Please note that a separate score is shown for each of the individual concerns (shown in parentheses under the Item number to which each of the individual concerns refers), to assist you in interpreting the review. An explanation of each of the levels of concern is shown above, in the chart on the previous page. The IACUC must review each of the **level 1** concerns listed 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, but no further correspondence with the CVMO is needed.

In case of questions about this review, please contact Dr. [redacted], Assistant Chief Veterinary Medical Officer at [redacted] or [redacted].

REVIEWER FEEDBACK

ACORP Item number(s) (score)	Comments/Concerns
ACORP (canine)	The ACORP uses pet (privately-owned) dogs with melanoma to determine if a new intratumoral immunotherapy in combination with radiation therapy and antibody therapy is a safe and an effective treatment. Dogs participating in the study will undergo treatment at the University of Wisconsin - School of Veterinary Medicine. Commendable aspects of this protocol include a sound justification for the canine melanoma model, the potential of this new therapy to benefit both human and canine melanoma patients, the highly skilled and experienced research staff, and the detailed procedural descriptions. A few concerns were identified. 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 and the revised ACORP must be reviewed and cleared by the CVMO before review and final approval by the Secretary of the VA.
Item E (0)	All study participants including the (to be identified) oncology clinical intern must complete the appropriate animal research training before beginning work on this study, please contact the CVMO for additional information.
Item G (1)	All participants in a VA supported study must be offered the opportunity to participate in an occupational health and safety program (OHSP). The table found in item G should be revised to clearly indicate whether each participation has declined or accepted OHSP enrollment.
Items T (1)	An understanding of adverse events grading for veterinary oncology studies would be improved if the Veterinary Cooperative Oncology Group-Common Terminology Criteria for Adverse Event (VCPG-CTCAE) grades were detailed in the ACORP, please add the information shown below.

(cont.)

	Grades Grade refers to the severity of the AE. The VCOG-CTCAE displays Grades 1 through 5 with unique clinical description of severity for each AE based on this general guideline: Grade 1 Mild; asymptomatic or mild symptoms; clinical signs or diagnostic observations only; intervention not indicated. Grade 2 Moderate; minimal, outpatient or non-invasive intervention indicated; moderate limitation of activities of daily living (ADL). Grade 3 Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; significantly limiting ADL. Grade 4 Life-threatening consequences; urgent interventions indicated. Grade 5 Death related to AE.
Appendix 5 (1)	Item 2 of this appendix indicates that the skin edges of the biopsy site will be closed with nylon sutures; please indicate the time frame in which the sutures will be removed.

(cont.)

CVMO Review Suggestions

ANIMAL COMPONENT OF RESEARCH PROTOCOL (ACORP)

Main Body

Proposal Overview

A. 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 10,000 people a year in the US. 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 an even higher rate of melanoma 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.

Unfortunately, once melanoma spreads 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 is so similar to human melanoma. If it works well in this study, we will then do full clinical trials in both dogs and humans.

B. Experimental Design.

1. **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 Wisconsin Veterinary Care (UWVC) 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 UWVC 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.

Part 1 of this study tests a new immunotherapy drug called "hu14.18-IL2" 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 brain cancer 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 brain cancer study. At each dose we will take blood samples 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 hu14.18-IL2 from part 1, combined with radiation therapy directed at the tumors. 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 the same amount 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 to determine which radiation therapy results in the stronger immune system activation, and monitor for side effects.

Part 3: We know tumor cells produce chemicals that inhibit the immune system, so we will add a drug called "anti-PD1 antibody" which will prevent the immune system from being inhibited. This drug will be combined with the best hu14.18-IL2 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. All the tumors will be injected with hu14.18-IL2 and treated with radiation. The anti-PD1 antibody 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. Some of the tumors will be treated with hu14.18-IL2 and radiation, and the anti-PD1 antibody 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.

2. **Complete description of the proposed use of animals.** Detail the proposed use of animals:

a. **Summarize** the design of the experiment in terms of the specific groups of animals to be studied.

► The primary goal of this study is to determine whether intratumoral immunotherapy with hu14.18-IL2 (IT-IC) in combination with local radiation therapy (RT) and immune checkpoint blockade is safe and has antitumor activity in canine melanoma. Dogs in the proposed study will be privately owned pets with spontaneously occurring melanoma. Exploratory studies will: 1) evaluate T cell responses in the blood and tumor before and after this immunotherapy, and; 2) utilize novel immune monitoring to identify a candidate biomarker of response for dogs with melanoma receiving IT-IC. In addition, blood samples will be collected from healthy pet dogs that do not have melanoma to be used as controls for flow cytometry and T cell receptor (TCR) repertoire analyses.

Part 1 (Aim 1a): We will initially study a low, medium, and high dose of IT-IC in 9-18 dogs with locally advanced or metastatic melanoma and will identify a maximum tolerated dose (MTD) or maximum administered dose (MAD) of IT-IC using a 3-day administration schedule. The doses used will not exceed that found to be safe in human pediatric neuroblastoma patients.

We recently completed preliminary safety testing, using separate funds, of the first two proposed dose levels of hu14.18-IL2 in tumor-bearing dogs at UWVC (Protocol Title: "Phase I Dose-Finding Trial of IT-IC in Tumor-Bearing Dogs", IACUC approval number [redacted]). These doses are lower than doses previously given intravenously to human pediatric neuroblastoma patients. The treatment was well tolerated by the treated dogs, however, only safety data, not biological data, were collected. Therefore, we will describe the study as designed but will allow for modification of cohorts and/or doses once the study is approved.

Part 2 (Aim 1b): Once the MTD or MAD is determined in Part 1, we will study another 12 dogs with locally advanced or metastatic melanoma to evaluate the safety of IT-IC at the MTD or MAD combined with RT to the local site, the same tumor site receiving IT-IC, in order to enhance its function as an *in situ* vaccine. The RT will be given either in a single 8 Gray (Gy) fraction or in three 8 Gy fractions and we will determine whether a single 8 Gy fraction or three 8 Gy fractions of RT merit subsequent testing with IT-IC in canine melanoma.

Part 3 (Aim 2): After completion of Aim 1, we will enroll another 12 dogs with locally advanced or metastatic melanoma into Aim 2 to determine safety, tolerability, and antitumor activity (based on clinical measurements as well as histological data) of the combination of local RT, IT-IC and anti-PD1 antibody. We will administer a caninized anti-canine-PD1 antibody (hereto referred to as 'anti-PD1') that has been designed to be recognized as "self" by the canine immune system. We will evaluate mechanisms of antitumor activity and will determine whether histologic findings of concomitant immune tolerance seen in our murine model are also present in the dog.

Blood samples and tumor biopsies will be obtained in Aims 1 and 2 for immune monitoring in Aim 3. This study has potential for high clinical impact as study findings in the dog will inform clinical development of IT-IC in human melanoma patients.

b. **Justify the group sizes and the total numbers of animals requested.** A power analysis is strongly encouraged; see ACORP instructions.

► Dr. [redacted], biostatistician collaborator on this VA Merit grant, has reviewed the study design. Since the study was originally designed and submitted, we have obtained pilot safety data from a Phase I dose-escalation study (IACUC approval number [redacted]). However, as biologic data was not collected in the Phase I study, the exact effect of the candidate biomarkers are not known. The primary outcome for the dose finding parts of the study is toxicity, whereas the primary outcome for the subsequent parts of the study is the determination of candidate biomarkers of response to the treatment.

The planned sample size for this study is between 38-47 dogs with melanoma (Part 1, Aim 1a: 9-18 dogs; Part 2, Aim 1b: 12 dogs; Part 3, Aim 2: 12 dogs); and 5 pet control dogs.

- Part 1 (Aim 1a).
  A total of 9-18 dogs (estimate 12 dogs) will be enrolled. As this is a dose escalation study with the primary objective to determine the MTD/MAD of IT-IC when given daily for 3 days to dogs with canine melanoma, no formal power calculations were conducted. Rather, the sample size chosen is based on a typical "3+3" dose escalation schema with 3 planned for each dose cohort and expansion to 6 dogs when indicated. The total number of dogs treated for this part of the study will depend on the number of dogs treated in each cohort before the MTD/MAD has been determined. It is expected that a total of approximately 12 dogs (9-18) will be required to complete the dose escalation.

- Part 2 (Aim 1b).
  A sample size of 6 dogs per treatment group (total of 12 dogs) will be enrolled in this Aim. This sample size will be adequate to detect anticipated moderate to large effect sizes with sufficient power when comparing candidate biomarker levels between arms. Specifically, a sample size of 6 dogs per arm will provide between 49-94% power at the one-sided 0.05 significance level to detect anticipated effect sizes ranging between 1.0-2.0 standard deviation units in candidate biomarker levels. Thus, the power would be 94% at the one-sided 0.05 significance level to detect an effect size of 2.0 standard deviation units in candidate biomarker level. Due to the exploratory nature of this study, there will be no multiple testing adjustments for evaluating multiple candidate biomarkers.

- Part 3 (Aim 2).
  A sample size of 6 dogs per treatment group (total of 12 dogs) will be enrolled in this Aim. This sample size will provide 70-99% power to detect a moderate effect size of 1.0-2.0 standard deviation units for the change in biomarker levels of T cell response to melanoma from the baseline to the RT, IT-IC, and anti-PD1 antibody post treatment assessments at the one-sided 0.05 significance level. Furthermore, large effect sizes of >2.5 for the differences of changes from baseline in biomarker levels between groups will be detected with 90% power at the two-sided 0.0167 (=0.05/3 – a Bonferroni adjustment for multiple comparisons between two groups). The study does not provide adequate power for detecting the anticipated correlations between changes in biomarker levels of T response and tumor expression of GD2 within each group. However, across the two groups, a moderately strong correlation of 0.6 or greater will be detected with 80% power at the two-sided 0.05 significance level.

c. **Describe each animal procedure** to be performed on this protocol. (Document in Appendix 9 any of these procedures that involve "departures" from the standards in the *Guide*. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)

► Information on recruitment, inclusion criteria, blood collection from controls, and pretreatment evaluations are included in Appendix 1.

Collection of Blood Samples from Dogs without Melanoma to Serve as Assay Controls

- A blood sample at one timepoint will be collected from 5 privately-owned dogs upon the owner's consent.

Treatment for Pet Dogs with Melanoma

- *Part 1 (Aim 1a) Schedule*: Dose escalation study to determine MTD or MAD of IT-IC.
  - This dose escalation study will include 3 dogs/cohort, but will allow for expansion of each cohort to 6 dogs/cohort if dose-limiting toxicity (DLT) is seen in one dog in the planned 3 dogs in each cohort (estimate 12 dogs total for the dose escalation part of the study). The IT-IC will be administered to the treatment site on days 1, 2 and 3. We will obtain tumor biopsies for analysis of melanoma tumor cells and tumor infiltrating lymphocytes (TIL) as well as peripheral blood mononuclear cells (PBMC) for immune monitoring at baseline and at various times post-injection. The dose levels planned for testing are based on our prior studies involving intravenous administration of hu14.18-IL2 in adults with melanoma and in children with neuroblastoma. The treatment groups to be studied are:
    - hu14.18-IL2 (2.0 mg/M2/day) x 3 days
    - hu14.18-IL2 (6.0 mg/M2/day) x 3 days
    - hu14.18-IL2 (12.0 mg/M2/day) x 3 days

- *Part 2 (Aim 1b) Schedule*: Determine localized and systemic toxicity of IT-IC at the MTD or MAD when given daily for 3 days following RT to dogs with canine melanoma.
  - The MTD or MAD of IT-IC from Aim 1a will be combined with RT in Aim 1b for dogs (6 dogs/cohort) with locally advanced melanoma. The RT will be delivered in a single 8 Gy fraction or in three 8 Gy fractions over 1 week (i.e., Day -10, Day -8, Day -6) to the primary site and regional lymph nodes when clinically involved (locally advanced melanoma) approximately 6 days or between 10 and 6 days, respectively, prior to IT-IC. The tumor will be biopsied pretreatment as well as 1, 2, and 3 weeks after IT-IC. The treated tumor will be left in place for 3 weeks in all dogs to allow it to function as an *in situ* vaccine. If dose limiting toxicity is seen in 2 dogs, 6 additional dogs would be entered at a lower dose of IC, or lower dose of RT, depending on which seemed likely as the cause for the DLT. Safety data will be reviewed and a dose reduction will be considered in the unlikely event that DLTs are observed in more than 2 of the initial 6 dogs.
  - Blood will be collected for immune monitoring pretreatment and various times post IT-IC treatment.

- *Part 3 (Aim 2) Schedule*: Determine the safety and tolerability of the combination of local radiation, systemic anti-PD1 antibody, and IT-IC in dogs with locally advanced or metastatic melanoma.
  - The MTD or MAD of IT-IC combined with RT from Aim 1b will be combined with anti-PD1 antibody. A total of 12 dogs with locally advanced or metastatic melanoma will be studied in this Aim in one of the following 2 groups:
    - Group A: 6 dogs with locally advanced or regional melanoma, but without distant metastases will receive IT-IC + RT treatment to all sites of tumor, in combination with anti-PD1 antibody.
    - Group B: 6 dogs with locally advanced or regional melanoma, and also with distant metastases, will receive IT-IC + RT treatment to the locally advanced or regional melanoma, but no RT treatment to distant metastases, in combination with anti-PD1 antibody.
  - We will obtain tumor biopsies for analysis of melanoma tumor cells and TIL as well as PBMC for immune monitoring at baseline and at various times post-injection.

Patient Follow-Up (Melanoma Dogs only)

- A physical examination including tumor measurements will be performed at each study visit.

CVMO Review Suggestions

- Tumor biopsies will be collected on days 10, 17, and 24. CBC, chemistry profile, and urinalysis will be performed on days 10 and 24. On days 10, 30 and 60, 12 ml of blood for immune assays will be collected and thoracic radiographs will be repeated on Day 30.

Treatment and Evaluation Schedules for Parts 1-3 (Aims 1 and 2 only; no dogs are treated in Aim 3)

C. 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 "Anim al Use Alternative s" 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, anti-PD1	(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)	( )
PubMed	3/6/18	All years available	N/A	canine melanoma, anti-PD1, hu14.18-IL2	( )	( )	( )	(X)
PubMed	3/6/18	All years	Cancer therapy adverse	Adverse events and anti-			(X)	

		available	events	PD1 treatment, adverse events and hu14.18-IL2 treatment, adverse events and radiation therapy				

Please use the **Animal Research Alternatives and Animal Care Guide** for literature searches to demonstrate the search for alternatives to using animals in research and ways to minimize painful procedures: http://researchguides.library.wisc.edu/animalalternatives

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 anti-PD1" 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 anti-PD1), 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 hu14.18-IL2.

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. [redacted], biostatistician at the University of Wisconsin to determine the minimum number of animals to be used. Please see section C2b for details.

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 UW 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 UW 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. [redacted], 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, anti-PD1, hu14.18-IL2 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.