Virginia-Maryland College of Veterinary Medicine
VIRGINIA-MARYLAND COLLEGE OF VETERINARY MEDICINE
ANIMAL CANCER CARE AND RESEARCH CENTER
VIRGINIA TECH

Clinical Research Project
Client Consent Form

Study Title: HeEV: Histotripsy-enabled Extracellular Vesicle characterization in canine soft tissue sarcoma patients

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One of the missions of the Virginia-Maryland College of Veterinary Medicine is to create, disseminate and apply medical knowledge through discovery, learning, and engagement. You are invited to participate in this mission by enrolling your animal in a clinical research study. Your participation is voluntary, and you may withdraw your animal from the study at any time by notifying the Principal Investigator. There is no penalty if you choose not to participate.

Study Purpose:

Soft tissue sarcomas are a common form of skin cancer in dogs. They tend to develop slowly and don't usually metastasize when they are low grade. A biopsy is needed to determine the tumor grade. If a minimal surgery (removal of only the tumor that can be seen or felt) is performed, the tumor is likely to grow back. Large surgery is the treatment of choice, but can be impossible without amputation or the addition of radiation therapy when the tumors are very large or affect certain areas of the body like the limbs or face. Alternative treatments to surgery or radiation are needed.

We have previously evaluated a non-surgical, non-radiation, non-thermal focused ultrasound treatment to kill soft tissue sarcomas affecting pet dogs. This technology has also been used in humans with liver tumors. **Histotripsy** focuses microsecond-long soundwaves through the skin onto a precise target area of the tumor. This causes air bubbles in the tumor tissue to expand and collapse, mechanically disintegrating the surrounding tumor tissue. Research in rodents, pet dogs, and humans have indicated that the liquified tissue stimulates the immune system to recognize this cancer in other parts of the body.

The purpose of this study is to evaluate one type of communication signal released from cells, termed **extracellular vesicles (EVs)**. EVs carry protein and genetic messages that immediately change the receiving cell. EVs and their cargo can be used as a non-invasive way to diagnose a tumor or look for signals to predict response to treatment or prognosis. We will extract and measure EVs and their immune signal cargo from the liquified tumor tissue, the lymph nodes, and the blood after histotripsy treatment of soft tissue sarcomas. We hope to find that histotripsy increases the number and type of EVs in the blood, which would be the first step in developing a "liquid biopsy" for diagnosis; we also hope to find that histotripsy treatment changes the communication with the immune system, serving as a mechanism to explain the changes seen in the immune response after histotripsy.

To determine how histotripsy may affect EVs, we are recruiting dogs with soft tissue sarcomas located in the skin to undergo histotripsy treatment of the tumor. ***An existing tumor must be present for treatment***.

The application of this experimental therapy has the potential to directly enhance the quality of life of dogs diagnosed with soft tissue sarcomas and defray the cost of treatment. At the same time, the results of our study may lead to improved liquid biopsy and treatment options for human cancer patients.

Study Design/Procedures:

Pretreatment evaluation (screening, prior to enrollment)

Before beginning treatment your dog must undergo the following to determine if s/he is a good candidate to enroll in the study. Numbers 2-4 can be completed by your family veterinarian and must be within the past 2 weeks. Numbers 1 and 5 must be performed with Oncology at ACCRC within 10 days of histotripsy treatment.

1. **Complete physical exam**
2. **Lab work:** We will obtain blood samples from your pet for a complete blood count and chemistry profile. Six milliliters (about 1 teaspoon) of blood will be collected from a vein in your pet's neck, which is the easiest access point. We will also collect 5 milliliters of urine.
3. **Biopsy of the tumor:** A needle sample, core or wedge sample of the tumor will be obtained for diagnostic purposes.
4. **Thoracic radiographs:** Three-view chest x-rays read by a board-certified radiologist are required before anesthesia.
5. **Tumor, thoracic, and abdominal imaging:** A CT scan of the thorax, abdomen, and tumor once the initial lab work,

diagnosis, and chest x-rays have been approved by the clinical trial doctor(s). The CT scan is required for histotripsy and treatment planning.

Eligibility criteria

To be eligible for enrollment:

1. Your dog must have a diagnosis of a soft tissue sarcoma located within the skin and measures at least 3 cm (1.2 inches) but no more than 18 cm (7.1 inches) in diameter.
2. Your dog must weigh at least 5 kg (11 lb), be healthy enough to undergo anesthesia during the study, and is expected to live for at least 4 weeks.
3. Your dog must have an existing tumor (new or regrowth after surgery)
4. It must have been at least 3 weeks since treatment with any anti-tumor systemic therapy, or 4 weeks since treatment with radiation.
5. You must be willing and able to complete all scheduled study visits regardless of treatment group assignment.

Treatment: The study visits are summarized in the figures on the next page.

There are two treatment arms in this study. Once your dog is enrolled, he or she will be randomized to either group A (one histotripsy treatment) or group B (three histotripsy treatments). Differences between treatment groups are in *italics*.

Group A: There are *(6) required in-clinic visits, (1) anesthesia procedure, (1) histotripsy treatment procedure, (1) tumor needle aspirates*, (6) lymph node aspirates, (9) blood draws, and (1) CT scan under sedation.
Group B: There are *(8) required in-clinic visits, (2) remote (zoom or phone) exams, (3) anesthesia procedures, (3) histotripsy treatment procedures, (3) tumor needle aspirates*, (6) lymph node aspirates, (9) blood draws, and (1) CT scan under sedation.

Histotripsy treatment: Once assigned to a treatment group the first histotripsy treatment will be scheduled within ten (10) days of the screening evaluation and enrollment. Histotripsy will be applied to the tumor under general anesthesia. Your dog's fur/hair will be clipped over the tumor as is standard for any ultrasound in dogs. Markings will be made on the skin overlying the tumor to indicate where treatment has occurred. These markings must remain in place for the duration of the study. The actual treatment will be tailored to each dog's individual tumor, and be delivered in a series of microseconds-long bursts of sound focused on the tumor to kill the cancerous cells through unbroken skin surface. The histotripsy treatment is expected to take 30 minutes to an hour, but may take longer for some patients. Histotripsy is not painful and does not use heat.

Samples for research purposes: A needle sample of the liquified tumor after each histotripsy treatment, 1 for dogs in group A and 3 for dogs in group B, will be collected for later analysis of EVs. Multiple blood and lymph node samples will be taken before and after treatment over multiple days for later evaluation of immune cells and EVs. It is not part of the study for your dog to stay overnight after any visit. Due to timing and for the safety of your pet, s/he may need to stay overnight while recovering from anesthesia. Your dog will need to return to the Animal Cancer Care and Research Center in Roanoke for blood +/- lymph node sample collection according to the schedule below.

CT scan post-treatment: Dogs in both treatment groups will undergo a CT scan of the tumor 14 days after the first histotripsy treatment. Your dog will be heavily sedated and will go home once she or he is awake. Some dogs may need general anesthesia based on the doctor's assessment. This CT scan can also be used for treatment planning for either surgery or radiation treatment to begin once the study is over.

Follow-up

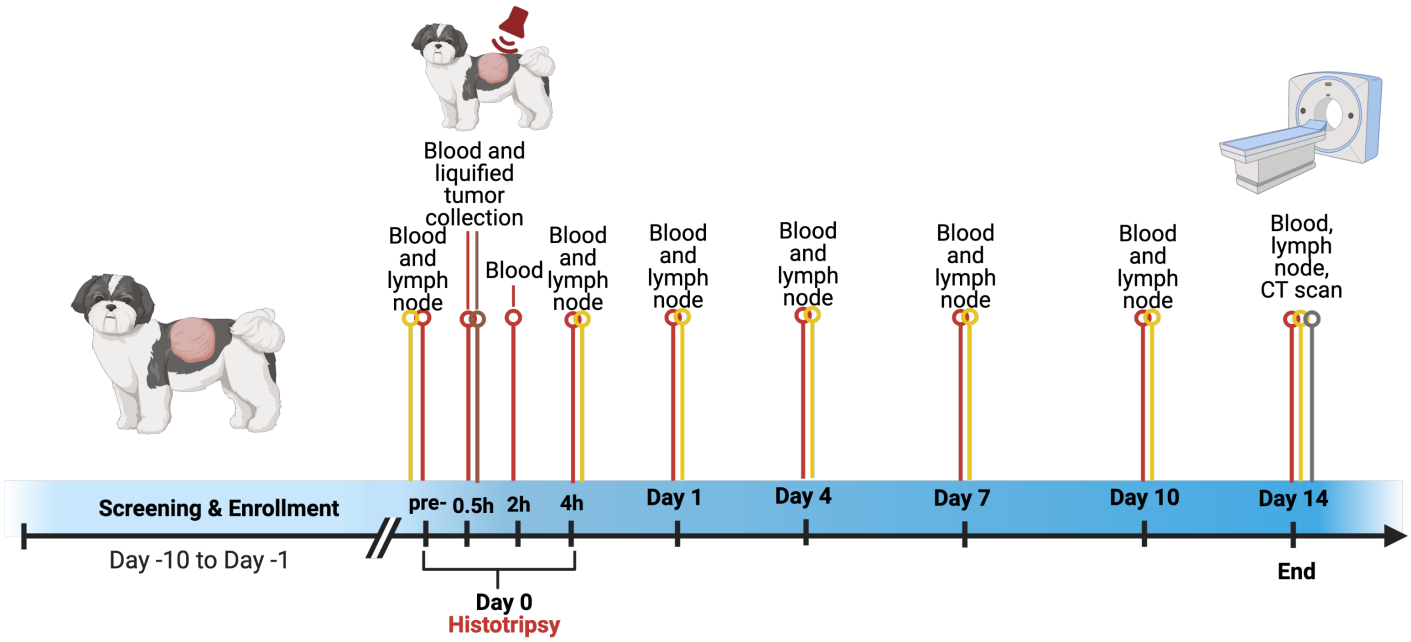
Your dog will not need to return to the Oncology service for any study-related procedures after completing the final study visit on day 14. We ask that you keep the clinical oncology service and investigators informed of your contact information and the status of your pet's health, as we will contact you and/or your regular veterinarian for follow-up information when the study enrollment closes.

Risks and Benefits:

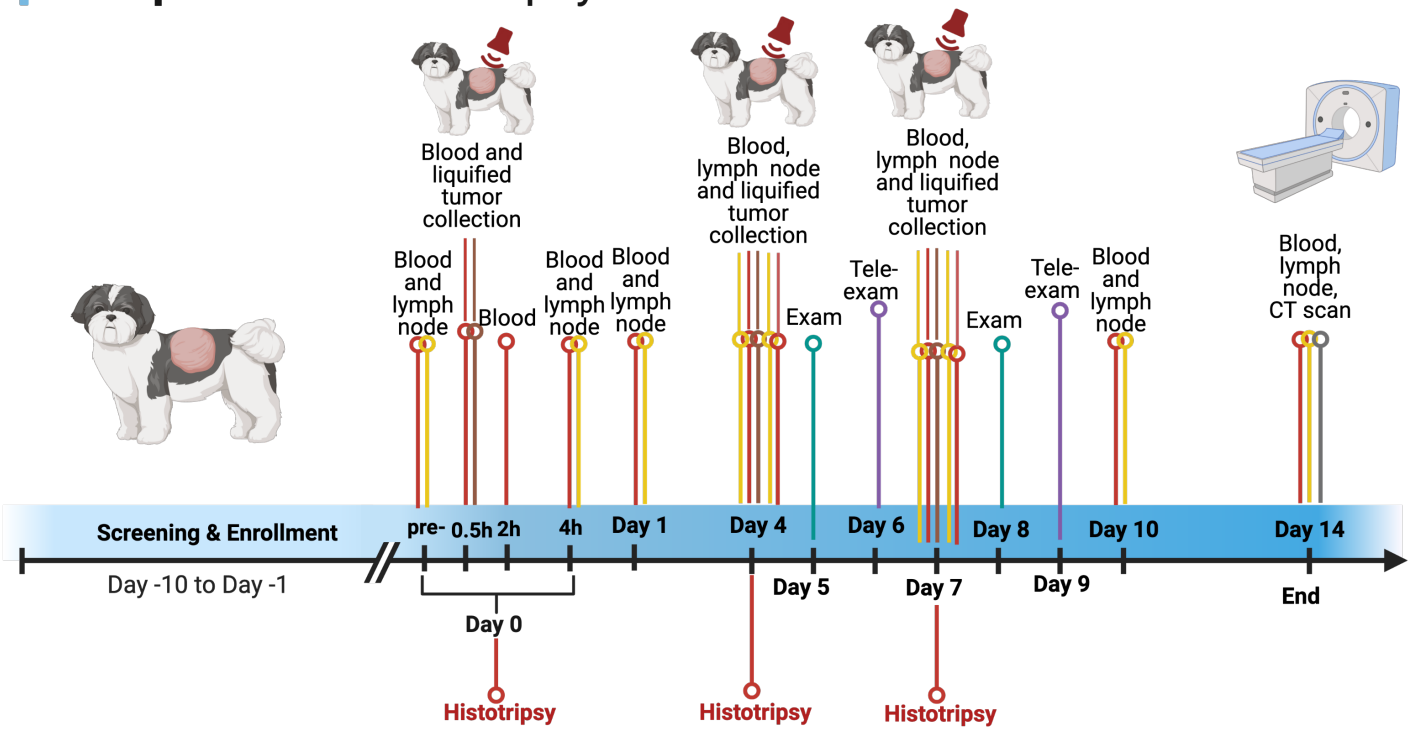
The goal of this study is to develop a new, more effective and safe therapy against cancer. Some of the procedures performed in this study are routine clinical procedures. **The histotripsy treatment is experimental and not part of the standard treatment.** Side effects that may be seen in your pet during this study may include but are not limited to fever, tumor inflammation, systemic inflammation, and death.

Although unexpected, there could be problems with the diagnostic procedures required for enrollment (lab-work, staging, biopsy). These problems can be due to inflammation or infection and may result in bruising at the collection site. Additionally, all animals going under general anesthesia are in risk of adverse effects that may result in death. We take stringent measures to minimize these risks by taking steps to prevent contamination of the biopsy site, and monitor continuously the vital functions of your pet when under general anesthesia and during the recovery period.

Group A: Single histotripsy treatment



Group B: Three histotripsy treatments



Study Costs and Compensation:
The screening (eligibility) tests are not covered by the study. You are responsible for any clinical fees exceeding the costs outlined below, any fees associated with medical complications of the histotripsy treatment, and diagnostics or treatments performed not associated with the study or other medical problems that arise during the study. Your total out of pocket costs are estimated to be between $2500 and $3000.

Screening and baseline CT scan: Lab work, chest x-rays, and diagnosis of soft tissue sarcoma cost approximately $1,000 if performed at ACCRC. If performed by your regular veterinarian, they can be sent to ACCRC to begin the

screening process. Tests and images of insufficient quality will not be accepted. An exam and CT scan with the oncologists at ACCRC will be necessary to fully determine whether your dog is eligible to enroll. The cost of the CT scan will be out-of-pocket, $1500. Anesthesia costs exceeding the allowed study amount (listed below) will be your responsibility.

Study procedures covered by the study:
Once your dog is eligible and enrolled, the study will provide $4,000 worth of diagnostics, treatment, and medical care. *Specifically:* Up to $350 for general anesthesia for histotripsy treatments, the exam fees and study-required lab work once enrolled, the sedation and CT scan on day 14, and $2,000 towards treatment of your choice at ACCRC upon completion of all study visits.

Confidentiality:
The data collected in the course of this study is confidential. In any publication or presentation of the study data, we will not include information that would make it possible to identify a research participant. Research records will be kept in a secure location; only researchers will have access to the records. The study sponsors will have access to de-identified study data.

Statement of Consent: You will be given a copy of this form to keep for your records.

In giving my consent by signing this form, I acknowledge that I have been informed of the purpose and nature of this study and its associated procedures, as well as any possible side effects.

- I have read and understood the above information.
- I have been given the opportunity to ask questions and receive answers, and I voluntarily consent to participate in the study. I further certify that I am the owner (or duly authorized agent of the owner) of _____.
  (Animal's name)
- I understand that data, tissue, images, and fluid samples (e.g. blood, urine) collected from my animal will become property of the study investigators. Study investigators may choose to share these samples with research collaborators at Virginia Tech and/or outside collaborators/entities. Any samples or data/images shared will have any identifying information removed prior to sharing.
- A member of the study team may contact me after my animal has finished this study to collect follow-up information. This may occur several months to years following completion of the study.

Owner or Agent Signature: _____ Date: _____

Owner or Agent Printed Name: _____

Attending Clinician Signature: _____ Date: _____

Attending Clinician Printed Name: _____

Please don't hesitate to contact us if you have any questions or concerns about this study.
The research and procedures have been reviewed and approved by the Virginia Tech Institutional Animal Care and Use Committee and the Virginia-Maryland College of Veterinary Medicine Clinical Research Review Committee.

If you have any questions or concerns regarding the study and would like to talk to someone other than the researchers, please contact:

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