VIRGINIA TECH
ANIMAL CANCER CARE AND RESEARCH CENTER

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

Purpose

To evaluate the activity of a type of communication signal released from cells in dogs with soft tissue sarcomas after they are treated with non-invasive histotripsy ablation

Background

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. Surgery is the treatment of choice, but if a minimal surgery (removal of only the tumor that can be seen or felt) is performed, the tumor is likely to grow back. Removal of the tumor and several centimeters of normal-appearing tissue is required for complete removal. However, this can be impossible without amputation or the addition of radiation therapy, particularly when the tumors are 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 (histotripsy) 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 shown that the liquified tissue stimulates the immune system to recognize 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 cause immediate changes in 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.

Eligibility

- 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.
- Dogs must weigh at least 5 kg (11 lb.), be healthy enough to undergo anesthesia during the study, and be expected to live for at least 4 weeks.
- Dogs must have an existing tumor (new or regrowth after surgery)
- It must have been at least 3 weeks since treatment with any anti-tumor systemic therapy, or 4 weeks since treatment with radiation.
- You must be willing and able to complete all scheduled study visits regardless of treatment group assignment.

Study Design

This study takes place at the Animal Cancer Care and Research Center (ACCRC) in Roanoke, VA.

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).

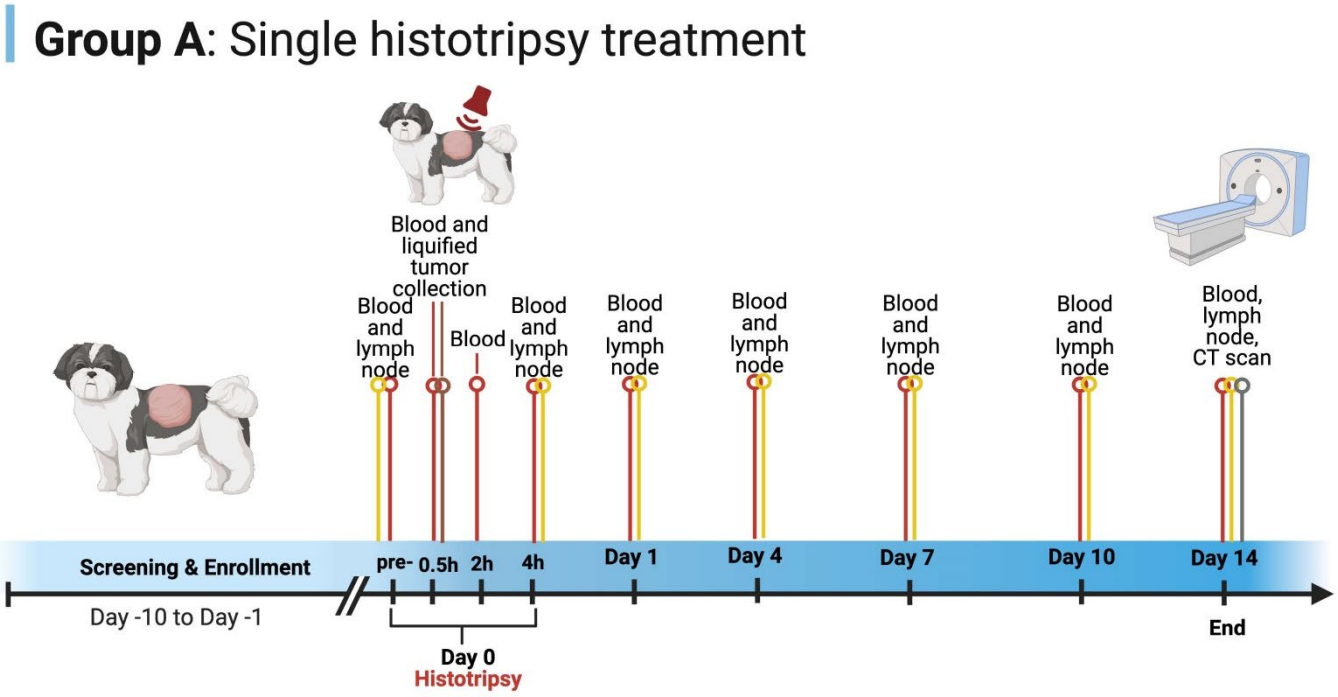
Group A: There are (6) required in-clinic visits, (1) anesthesia procedure, (1) histotripsy treatment procedure, (1) tumor needle aspirate, (6) lymph node aspirates, (9) blood draws, and (1) CT scan under sedation.

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. However, 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 schedules below, depending on their assigned group.

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.



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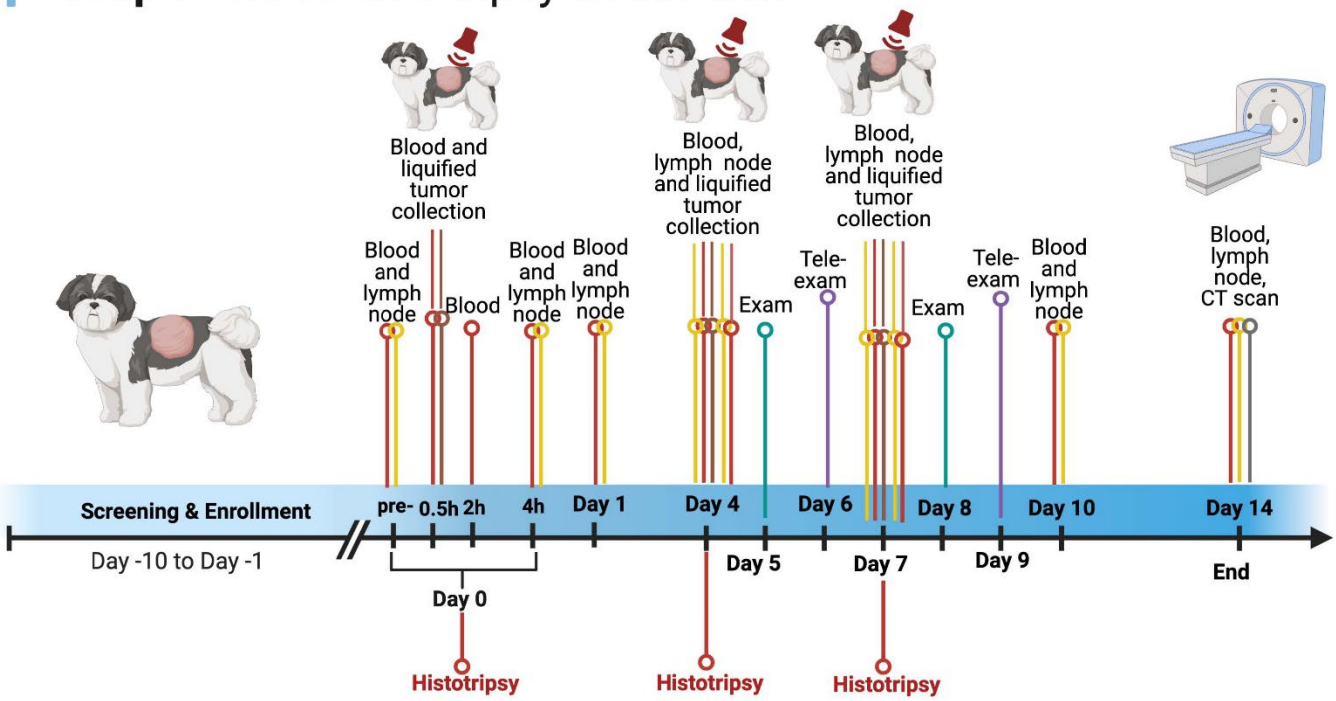
Group B: Three histotripsy treatments



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 $2,500 and $3,000.

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, results 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, $1,500. 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.

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