

PARTICIPANTS NEEDED FOR RESEARCH ON CANINE CANCER

THE STUDY

The research project “Cellular Genomics- A molecular cytogenetics investigation of canine soft tissue sarcoma” is part of Dr. Matthew Breen’s laboratory program “Genetics of Canine Cancer”. This project is supported by the American Kennel Club Canine Health Foundation along with several breed clubs and animal health organizations. The purpose of the project is to determine which regions of the canine genome are recurrently associated with the incidence soft tissue sarcomas, histiocytic malignancies and hemangiosaroma in the dog.

SCIENTIFIC APPROACH

It has been established that non-random chromosome aberrations are characteristic of specific types of many different human cancers. The knowledge of such aberrations has identified areas of the human genome to be targeted for further research. In the dog the extent and identity of chromosome aberrations associated with specific cancers is still largely unknown.

Soft tissue sarcomas in dogs are relatively rare, occurring at a frequency of approximately 1%. However, in certain breeds, such as the **Flat-Coated Retriever** and **Bernese Mountain Dog**, soft tissue sarcomas account for up to 50% of all malignant tumors and thus represent a serious health and welfare issue for those breeds. Histiocytic malignancies and hemangiosarcoma are also prevalent in these two breeds as well as **Golden Retrievers**, **German Shepherds** and **Briards**. These tumors are difficult to classify by conventional methods alone and so attention is required to develop improved modes of diagnosis. In humans sarcomas have been demonstrated to be associated with specific chromosomal aberrations that have been shown to have both diagnostic and prognostic significance. This CHF funded project is using major recent advances in canine molecular cytogenetics to identify recurrent chromosome aberrations associated with canine soft tissue sarcomas, in particular those of histiocytic origin. This project will identify areas of the canine genome associated with such cancers for further investigation at the sub-chromosomal level.

We are particularly interested in receiving tumor samples from Bernese Mountain Dogs, Flat Coated Retrievers, Golden Retrievers, German Shepherds, Briards and Australian Shepherds diagnosed with either histiocytic malignancy or hemangiosarcoma. These five breeds are referred to below as the ‘target’ breeds.

PARTICIPANT REQUIREMENTS

Dogs from one of the six target breeds with known pedigrees can participate in this study. Dogs from other breeds require prior approval from the principal investigator. The affected dog(s) can be male or female (intact or neutered), and must have a diagnosis of a soft tissue sarcoma, histiocytic malignancy or hemangiosarcoma, made by a licensed veterinarian. Owners of affected dogs will be required to sign an informed consent forms authorizing use of blood and tumor biopsy material for research purposes.

Necropsy samples may be submitted provided that they are taken and sent to Dr. Breen on the day of death and that the dog has not been receiving chemotherapy for their cancer.

Participation in this study will require the following materials to be submitted to NCSU.

MATERIALS REQUIRED – Cancer bearing patients

1. 5-10ml of blood collected into an EDTA (lilac top) tube(s) from the dog diagnosed with a soft tissue sarcoma/histiocytic malignancy/hemangiosarcoma
2. A biopsy of the tumor collected into formalin.
3. A biopsy of the tumor collected aseptically into sterile tube: ideally the tube will contain sterile transport medium, but in the absence of such medium, the sample may be sent in an empty sterile tube.
4. An official full pedigree of the individual diagnosed with the cancer (*this may be sent at a later date but will be required as part of the recruitment process*).
5. A copy of the pathology report associated with the diagnosis of soft tissue sarcoma (you should be able to obtain this from your veterinarian).

Your veterinarian may take all three samples during the normal procedures for diagnosis and treatment of cancer that are requested and approved by the owner of the dog.

MATERIALS REQUIRED – Relatives of cancer bearing patients

If you have been asked and agreed to submit a blood sample from a relative of a cancer patient, we ask that you provide the following:

1. A small (5-10ml for adults, 2ml for puppies) blood sample (EDTA/lilac top tube).
2. An official full pedigree of the individual showing the relationship of your dog(s) to the dog diagnosed with the cancer (*this may be sent at a later date but will be required as part of the recruitment process*).

As time progresses we ask that you keep us notified of any cancers that develop in your own dog(s) so that we may reconsider any data in the correct context.

MATERIALS REQUIRED – Puppy program

If you have been asked and agreed to submit a blood sample from a new puppy/litter, all you need to provide initially are the following:

1. A small (2ml) blood sample (EDTA/lilac top tube).
2. An official full pedigree of each puppy in the litter (*this may be sent at a later date but will be required as part of the recruitment process*).

As time progresses and the puppies mature, we ask that you keep us notified of any developing health concerns, especially cancers.

BreenLab - Molecular Cytogenetic Investigation of Soft Tissue Sarcoma
General information and sample submission requirements

The following steps must be followed precisely to facilitate enrolment and proper treatment of samples:

1. The owner and/or attending veterinarian must contact the lab of Dr. Matthew Breen, preferably by e-mail at info@BreenLab.org , or phone at 919-513-1466, to advise that a sample is being sent to the lab. **Ideally this needs to be at least 24 hours prior to the samples being taken.**
2. The owners/authorized agents of all dogs submitted must read and sign an informed consent form. This is available to be downloaded from the laboratory's web site at <http://www.breenlab.org/recruitment.html>. Please submit this completed form with the samples.
3. When sending a tumor sample, this must be obtained using aseptic technique (surgery or biopsy) by a licensed veterinarian using a **STERILE PACK**. This sample must be collected into an empty **sterile** tube
4. A sample of the tumor must also be placed in formalin. PLEASE NOTE: Samples cannot be received on Saturdays or Sundays and since samples will undergo degradation upon prolonged storage, we request that procedures performed on Fridays be avoided, whenever possible. If your samples are taken on a Friday, please store in a refrigerator (NOT a freezer) over the weekend and ship on Monday.
5. A blood sample (5-10 ml for adults, 2ml for puppies) must also be submitted in EDTA ('lilac-top' tubes) in an appropriate shipping container.

IT IS ESSENTIAL THAT ALL SAMPLES ARE RETURNED TO DR. BREEN'S LABORATORY BY OVERNIGHT DELIVERY WHEREVER POSSIBLE.

For additional information please contact Dr. Breen's lab by e-mail at info@BreenLab.org or by telephone at 919-513-1466 (office). We aim to return all calls/e-mails within 24 hours.

Flat-Coated Retriever owners may also contact the FCR Cancer Studies Support Team FCRCancer-Support@yahogroups.com

Bernese Mountain Dog owners may also contact Pat Long (BMDCA Health Committee member) at pat@bmdinfo.com

Briard owners may also contact Carol Hamilton (NC) at cmh2004@earthlink.net or Regina Keiter (Sr.) (PA) at luvbriards@gmail.com or Lynda Ronell (CA) at kings_oaks@earthlink.net

Golden Retriever owners may also contact Rhonda Hovan (GRCA Health Committee member) by telephone at 330-668-0044 or 330-338-4236 (cell) or by e-mail at RhondaHovan@aol.com

Australian Shepherd owners may also contact Kim Monti by telephone at 505.890.6150 or by e-mail at 4aussies_16paws@foxwoodkennel.com

INSTRUCTIONS FOR VETERINARIANS

Thank you for assisting your client in submitting valuable research samples that will be used to help us learn more about cancers in our dogs. Your help is very much appreciated. On the following two pages of this document we have provided detailed instructions to help you submit samples in the manner most suited to maintain their integrity during transit.

- Blood samples and tumor tissue will need to be submitted at the time of diagnosis.
- You should still submit a biopsy to your regular pathology service for a routine diagnosis. The study will **not** be able provide you with a diagnostic pathology report. Please either fax us a copy of the pathology report (919-513-7301, attn. Dr. Matthew Breen) or provide the owner with a copy of the pathology report to be sent to us.
- Please advise the owners that as part of the recruitment process we will also need a blood sample(s) (5-10ml from adults and 2ml from puppies) in EDTA/lilac top tube(s) from any available first-degree relatives of the dog diagnosed with a soft tissue sarcoma/histiocytic malignancy/hemangiosarcoma. These can come at a later date.
- We will require follow-up information on each patient, including specific treatment protocol, time to remission, disease-free interval, condition and time of relapse (if applicable), survival time, and cause of death. We will make every effort to contact you regularly to obtain information on this patient, and you may fax updates from the medical record to Dr. Breen at 919-513-7301.

For more information, contact the laboratory of Principal Investigator, Dr. Matthew Breen by telephone at 919- 513-1466 (lab) or by e-mail at info@BreenLab.org

SPECIFIC INSTRUCTIONS FOR SAMPLE SUBMISSION:

•**Peripheral blood samples:** we require two to three EDTA (lilac-top) tubes (2-5 ml each), mixed well from adult dogs, or a single 2ml tube from a puppy. These may be shipped by regular mail, and **MUST** be sent in a postal service approved crushproof container. Tubes must NOT be sent in padded envelopes. If blood samples are shipped within the same package as tumor samples (see below), please isolate the blood tubes in a sealed, impermeable (e.g., Ziplock) bag.

•**Non-fixed tumor samples:** we require fresh, **sterile**, viable tumor tissues for this study. It is imperative that tissues be obtained from viable areas, as necrosis will hinder our ability to use the samples. Sterile, viable tissue allows for the establishment of cell lines that can be used in the laboratory for the analysis of chromosome changes. Please submit the biopsy sample in an empty sterile tube (eg red top tube) – this sample **MUST** be shipped by overnight courier, with ice-packs, at the owner's expense. Please do not send tissues in snap seal bags or zip-lock bags.

Please label each tube with the origin of the tissue it contains. If biopsies are taken from different tissues, please put the tissues in different tubes and label accordingly. If samples are taken at necropsy please note the approximate time of death and the time of sample collection so we may determine the likely viability of the tissues submitted for investigation.

•**Fixed tumor samples:** in addition we also require a piece of the tumor(s) to be submitted in formalin so that we may perform our own investigative pathology at a later date. The formalin sample must be sent in a suitable screw cap formalin container. Please label each tube with the site of origin of the tissue it contains.

NOTE: for both the formalin tube and the sterile (+/- transport medium) tube, the minimum size for the biopsy is 5mm x 3mm x 3mm. Please do NOT send pieces larger than 2 inches.

SPECIAL HANDLING - PLEASE NOTE: Collection of viable tissue requires exceptional attention to detail and aseptic technique. The tumor tissue should be obtained with a **STERILE PACK** and placed into a sterile container. If the tumor sample is too large to fit in the container, it can be cut into smaller sections using sterile instruments while the tumor is placed on a sterile surface (for example, a sterile sponge or the surgical drape).

The tissue must never contact a non-sterile surface (for example, the operating table or the instrument table). Sterile saline (0.9% NaCl) is **unacceptable** as a transport media because the lack of a pH buffer makes the solution acidify rapidly, diminishing the viability of the tissue.

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General information and sample submission requirements

The container (tube or tubes) with the sterile tumor sample should be placed on ice (a suitable ice pack for shipping) immediately and shipped overnight e.g. via Federal Express/DHL, observing all regulations for shipping of viable tissues/biological specimens.

Please contact the lab in advance to advise that a parcel is being sent so that arrangements for timely sample processing can be made.

For more information, contact the lab of Principal Investigator, Dr. Matthew Breen, at 919-513-1466.

PLEASE SEND ALL SAMPLES TO:

**Dr. Matthew Breen
Soft Tissue Sarcoma Project
NCSU College of Veterinary Medicine
CVM Research Building
1060 William Moore Drive
Raleigh
NC 27607
Tel. 919-513-1466
Fax: 919-513-7301**

**Please clearly mark the package:
For immediate delivery to the CVM Research Building**

Thank you again for your help with this patient.