NUMBER 8 · MARCH 2024

LEONBERGER HEALTH FOUNDATION INTERNATIONAL Newsletter

The LHFI is excited to announce our support of the continuation of the EGFR/HER2 Immunotherapy Vaccine Trial! Pilot studies in this program indicate that incidence and/or stasis or reduction of metastatic masses can be achieved with EGFR based immunotherapy.

Evidence of the success possible with this therapy can be seen in the story of one of our own --

In 2020, Dr. Jen O'Keefe DVM's beloved Leonberger Digory was diagnosed with osteosarcoma. Knowing that osteo is a fatal disease and that time is of the essence should she choose treatment instead of palliative care, she moved quickly to research the available treatment options.

It was one of her colleagues, a Board Certified Veterinary Oncologist, who suggested she investigate an immunotherapy vaccine trial being conducted by Yale University. The information she read about the trial was promising and Digory was in the prime of his life so, armed with her veterinary knowledge and hope, she and Digory committed to the trial.

Read Digory's Story as told by Jen on Page 2.

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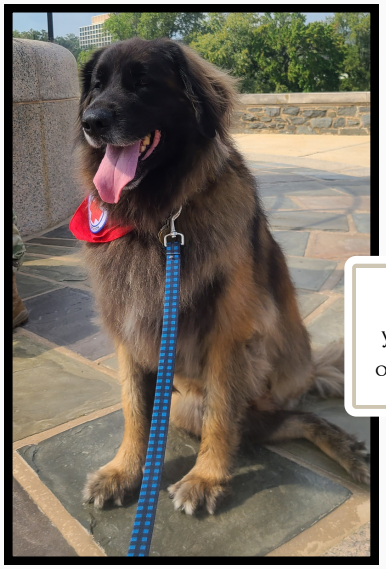


Digory's Trial

as told by his owner, Dr. Jen O'Keefe DVM

LHFI Board Member

With the help of the EGFR/HER2 immunotherapy vaccine, Digory gained 2 years and 10 months of a full and wonderful life after being diagnosed with osteosarcoma. All photos were taken post-amputation and during treatment.

One day in April 2020, I took Digory hiking as we often did. This was the beginning of the pandemic when everything was shut down. Hiking was one of the few activities that was still acceptable. Aslan's mobility was declining and I knew we wouldn't have long. Hiking was how I coped with everything, got my brain sorted out.

That April morning, we hiked about 4 or 5 miles, our normal route. The hike was pretty uneventful, and when we finished, Digory got into the back of the Jeep as always, and we headed home to get ready for work. It was about a 30 minute drive. When I went to get Digory out of the Jeep, he began crying out, not putting any weight on his left rear leg. Nothing had happened on our drive, so clearly I was concerned.

I gave Digory some pain medication we had for Aslan and got ready for work. When we got there, Digory was still not really using his leg, but at least he wasn't crying. At the end of my shift, we took radiographs of his leg. I was hoping for a torn ligament in his knee, or even a pulled muscle. But when I looked at the screen, I saw the destruction in the bone. My stomach dropped, and I knew it the worst-case scenario—Osteosarcoma. I left without saying a word, I couldn't speak.

The following day, I talked to one of our surgeons about our options. I knew without amputation, the pain would just get worse, and I couldn't do that to him. I'd seen many dogs with amputations do well, but I also knew the long-term prognosis was only 9-12 months. I struggled with the decision—was it fair to take his leg for such a short time? Was I doing it just for me?

Ultimately, after a long, sleepless night, I decided to go forward with amputation. Digory was scheduled for surgery the next day. Despite the anxiety I had making a choice, as soon as they rolled him into the operating room, a peace came over me. For better or worse, we were on a path and had a plan.

Digory's Trial

as told by his owner, Dr. Jen O'Keefe DVM

Surgery went well, and Digory's recovery went relatively smoothly. He was up and walking a couple days later. He resisted any assistance—he didn't want any help supporting his back end, so for the most part, I let him do his thing. The only thing he conceded to letting me help was to get up onto the bed. My bed is pretty tall, and he was a cuddler. After a couple days at home, he tried to jump up on the bed before I could stop him. He didn't make it, falling right on his surgery site. I think my heart stopped for a minute! He was fine, but afterwards, he would allow me to give him a boost onto the bed at night.

Once the biopsy came back confirming Osteosarcoma, we set Digory up for a course of chemotherapy--4 rounds of carboplatin 3 weeks apart. Digory was a trooper. As a therapy dog who had been furloughed since March, he was in his element, flirting with the Oncology nurses, who in turn showered him with treats and belly rubs. He adored his oncologist, Dr. Boostrom. Throughout the course of his treatment, he had the rare bout of diarrhea and skipped a single meal but had no other side effects.

After completing his 4th carboplatin treatment, there were still no signs of metastasis. I sat down with Dr. Boostrom to discuss the next step. By then, we had lost Aslan, and Digory and I were both taking it hard. Unfairly, I told Dr. Boostrom that he had to keep Digory alive because my heart couldn't go from 2 dogs to none in such a short time. I also told him that I wasn't emotionally able to make a decision—that he had to tell me the best option. I couldn't lose Digory, but I also couldn't let him suffer. Dr. Boostrom suggested a vaccine from Professor Mark Mamula at Yale. It was still in trials, but was showing promise in Osteosarcoma, and he thought it was worth trying. It was immunotherapy, helping the body to fight off cancer cells. He said that if Digory was his dog, that's what he would do. So that's what we did.

In August, 1 week after losing Aslan, Digory received his first dose of the Yale vaccine. He would get 2 doses, 3 weeks apart. The only real side effect was the possibility of sterile abscesses at the injection site, typically on the neck. Digory did develop golf ball sized abscesses at each injection site. When they ruptured, he got a funny haircut and wore a t-shirt to help keep him clean, but otherwise they bothered me more than they did him. The worst part for him was that it meant no swimming until they healed up.

The good news was that an abscess forms due to the response of the immune system and formation of antibodies, and typically means that the vaccine is working. Sure enough, when Digory had blood drawn a few weeks later, he had plenty of antibodies.

Even though he was doing well physically, Digory was still struggling with the loss of Aslan, who was his whole world. He enjoyed his walks and swims (once the abscesses healed), and time with his best buddy, Obi. But he still wasn't Digory. A new puppy was planned for January, which would be 9 months after his diagnosis. I was worried that he would give up and wouldn't make it that long. So over Thanksgiving, we brought home our "emergency" puppy, Caspian. Within days, Digory became Digory again. It was clear that Caspian was Digory's puppy, even after Rilian joined us in January.

Digory's Trial

as told by his owner, Dr. Jen O'Keefe DVM

Digory continued regular rechecks, including bloodwork and chest radiographs every 3-4 months. Shortly before each appointment, my chest would get tight until I again heard that we had the all-clear—no sign of the cancer coming back. Digory had no such anxieties—he continued to flirt with the nurses and buddy it up with Dr. Boostrom.

Through all of this, Digory was happy. That was the most important thing to me, that he was comfortable and still enjoyed life. When things began to open up after the pandemic, he resumed his job as a therapy dog. He was an inspiration to so many. Digory was able to connect with the Wounded Warriors we visited at Walter Reed in a whole new way, and taught school kids about living with disabilities.

Digory developed a partial tear in the cruciate ligament in his remaining leg, so our hikes turned into shorter, more leisurely walks. He loved to run and wrestle with his brothers. He would swim until he was exhausted, so I made him wear his life jacket. When we took a group of 7 Leonbergers to the beach, Digory was the first in the ocean and even kept up with everyone scrambling on the rocks. He also participated in the Belmar St. Patrick's Day parade. I was afraid it would be too long of a walk and didn't want him to be left out, so he was pushed in a stroller rather than pulling a cart as he did pre-COVID.

In February of 2023, Digory developed a slight limp in his left front leg. It didn't improve with conservative therapy, so we took him back for radiographs. There were concerning changes, although subtle, so before making any decisions, we took a biopsy. Unfortunately, it showed he had developed another tumor. Obviously, amputation was not an option this time. We talked about palliative radiation. The next stage of the Yale vaccine trials hadn't opened yet, but we talked about trying to get him a booster—they have since seen that antibodies decline over time.

The night before we were to start radiation, Digory let me know he was done fighting. He was restless and couldn't get comfortable. I knew I had to let him go. We cancelled his appointment, and he spent the day being spoiled. Dr. Boostrom came with all sorts of goodies, including chocolate cake with chocolate frosting. Obi came down from Pennsylvania to say goodbye. And my nieces, his "kids" came over for some cuddles. That night, February 27, 2023, I said goodbye and let him go.

The decision to amputate Digory's leg was one of the toughest choices I've had to make. If I hadn't been losing Aslan at the time, I don't know if I would have done it. But I have zero regrets. Digory got an extra 2 years and 10 months, at least 2 years longer than expected, thanks to the Yale vaccine. During that time, he lived his best life, happy and comfortable. And was loved by so many.

Details: EGFR Vaccine Trial Study for Osteosarcoma in Leonbergers

Provided by LHFI Board Member, Sandy Love
Liason to Lead Researcher Dr. Mark Mumula (Yale University/TheraJan)

The Leonberger Health Foundation International is supporting a new EGFR vaccine trial for the treatment of osteosarcoma in 30 Leonberger dogs. The vaccine is being developed by Dr Mark Mamula at Yale University School of Medicine. Epidermal Growth Factor receptors (EGFR) are highly expressed on the surface of canine osteosarcoma cells and have been found to be involved in promoting aggressive tumor growth. Immunotherapy targeting of these receptors has been successfully used to treat human cancers and is now being tried in dogs.

By the time osteosarcoma is diagnosed in a dog, cancer cells have already spread throughout the body. Even though the primary bone tumor is removed, these metastases will grow and eventually cause death. This is why osteosarcoma has such a poor prognosis. It is hoped that the new vaccine will induce antibody production against canine EGFR. These antibodies can then bind to EGFR on the tumor cells and block EGFR signaling. If successful, growth of metastatic cancer cells will then be slowed or eliminated.

Currently, treatment for osteosarcoma includes surgical removal of the primary bone tumor (amputation or limb-sparing procedures) and chemotherapy. Since bone cancer is one of the most painful cancers and is resistant to pain medications, a decision to euthanize or treat must be made as soon as possible. Your vet can help with this decision as there are a lot of things to consider; dog's physical health, location of tumor, risks/benefits, possible complications, cost, time commitment for travel and recovery, home situation (e.g. stairs), etc. Unfortunately, despite treatment, lifespan is only extended by a matter of months (median of 10 months) due to growth of the metastatic tumors that have already spread. Preliminary studies have found that addition of the new EGFR vaccine can help reduce and/or eliminate these metastatic tumors, extending survival times by 6 months or more over those obtained by standard of care treatment alone.*

If you wish to participate in the vaccine study, you will need to contact one of the oncologists on the list. They will help coordinate treatment with a surgeon. Because this is a research study, only sites on the list which have been qualified by the USDA can be used. There is a study protocol that needs to be followed. It will be very important to collect tumor tissue and blood samples at the time of surgery. In addition, radiographs, or other tests (e.g. ultrasound, urinalysis) will be required to help predict which cells or molecules may be predictive of an immune response. Your dog will receive an initial dose of vaccine and then a second booster at 21 days. Follow-up testing will include chest x-rays at 3, 6, and 9 months. Additional blood work will be collected at each visit; baseline, day 21, day 40-50, 3 months, 6 months, and 9 months. Your dog will be followed for at least 12 months. There is no cost to the owner for the vaccine, but you will be responsible for the cost of the evaluations, surgery, and chemotherapy.

*H Doyle, R Gee, T Masters, C Gee, C Booth, E Peterson-Roth, R Koski, S Helfand, L Price, D Bascombe, D Jackson, R Ho, G Post, M Mamula. Vaccine-induced ErbB (EGFR/HER2)-specific immunity in spontaneous canine cancer. Translational Oncology 14 (2021) 101205.

EGFR Osteosarcoma Vaccine Trial Sites

***** Indicates sites that have been confirmed to offer both amputation and limb-sparing procedures, or work with surgical groups that do.

Chelsea Tripp, DVM DACVIM (Oncology)
Bridge Animal Referral Center
8401 Main St.
Edmonds, WA 98026
425-697-2272

Rance Sellon, DVM DACVIM (Oncology)
Washington State University
Department of Veterinary Clinical Sciences
Pullman, WA 99164-6610
509-335-0711

Karina Valerius DVM DACVIM (Oncology) and
Jeanne Lane, DVM DACVIM (Oncology)
MedVet Cincinnati
3964 Red Bank Rd.
Fairfax, OH 45227
513-561-0069

Betsy Hershey, DVM DACVIM (Oncology)
CVA Integrative Veterinary Oncology *
2501 N 32 nd Street
Phoenix, AZ. 85008
602-841-0626

Roberta Portela, DVM DACVIM (Oncology) and
Mark Byrum, DVM DACVIM (Oncology) *
MedVet Chicago
3305 N. California Ave.
Chicago, IL 60618
773-281-7110

Brendan Boostrom, DVM DACVIM (Oncology)
MedVet Northern Virginia *
8614 Centreville Rd.
Manassas, VA 20110
703-361-8287

Katie Wright, DVM, ACVIM (Oncology),
MedVet Salt Lake City
331 W. Bearcat Dr.
Salt Lake City, UT 84115
385-341-4444

Rochelle Prudic, DVM, ACVIM (Oncology),
MedVet Cleveland
20400 Emerald Pkwy
Cleveland, OH 44135
216-362-6000

Christine Anderson, DVM ACVIM (Oncology),
MedVet Pittsburgh
2810 Washington Rd.
McMurray, PA 15317
724-717-2273

Dr. Audrey Chouinard, DVM (Oncology)
Omemee Veterinary Hospital *
128 King Street West, Ontario CANADA K0L2W0
705-799-5228

Melissa Miller, DVM (Oncology *
6506 W. Broad Street
Richmond, VA. 23230
804-206-9122
[Surgery: Dr Barnes, James River Vet Surgery (804-650-0151)]

CHF Grant Number	03032-MOU
Project Title	Early Detection of Canine Osteosarcoma
Institution	University of Minnesota
Investigator(s)	PI: Jaime F. Modiano Co-Is: Antonella Borgatti; Brenda J. Weigel; Logan Spector; Daniel Vallera; Aaron Rendahl
Project Start Date	04/01/2022
Report Number	Mid-Year 2
Current Date	10/31/2023

Update!

MODIANO/CHF STUDY: EARLY DETECTION OF OSTEOSARCOMA

In this study, we aim to identify blood biomarkers that can be used to assign a probability of developing osteosarcoma to otherwise healthy dogs at risk for this disease. We have a large set of samples and are also collecting new samples for this project.

The control (old and new) samples include healthy dogs and dogs with cancer. These samples are used to identify the patterns that are exclusively present in dogs with cancer, and specifically in dogs with bone cancer. The experimental group includes healthy dogs at risk for development of bone cancer. This risk is based on age (older than 4.5 years) and on size. Dogs from six breeds are receiving preference in recruitment, although large dogs of other breeds and mixed breeding are also eligible to enroll.

A gift from the Irish Wolfhound Foundation in advance of this project was instrumental in helping us to finalize the infrastructure. It also allowed us to pilot enrollment of 25 dogs to make sure the protocols were efficient and reasonable. Recruitment for the experimental group was launched at the end of August of 2022 and we began enrolling dogs and receiving samples, in random order, in October 2022.

As of October 31st, 2023, we have collected 434 new samples for both parts of this project. We estimate enrollment will be complete by the end of 2023 or in early 2024, at which time follow-up will continue for the duration of the project (and ideally for the lifetime of the dogs) and analysis of the data will begin to construct the metrics of the test. Such a test for predicting the occurrence of bone cancer in dogs would be extremely valuable to veterinary medical community and dog owners world-wide. Our approach will use artificial intelligence technologies to describe patterns that are associated with the formation of these cancers.

THIS STUDY IS SUPPORTED BY THE 2021 LHFI OSTEOSARCOMA MATCH CAMPAIGN

Update from Katie Minor

University of Minnesota

Canine Genetics Laboratory

DNA Bank & Sample Database: Our DNA repository and medical record database, combined with the University of Bern, now contains >11,500 Leonberger DNA samples.

At the University of Minnesota we currently have ~330 buffered blood samples and 295 cheek swab samples submitted for testing and/or research in previous years that we would like to fully process to archive the DNA. These samples were submitted prior to our current protocols that fully extract all samples received upon initial submission. In 2023 we completed fully processing ~200 hundred samples.

Genetic Data: A new 14.5 Tb external hard drive was purchased to upgrade and replace the aging hard drives that house the Leonberger data at University of Minnesota. During the coming year we will be verifying that all data is backed up to at least 2 locations to prevent data loss in the event of hard drive/server failure.

	SNP arrays	Whole Genome Sequencing
Leonberger	1494	87
Saint Bernard	116	15

Additional SNP array data should be delivered within the next 1-2 months for the University of Zurich's glaucoma research (8 cases), as well as cases for unexplained neuropathy (3 cases).

The University of Minnesota completed a new build of our canine whole genome sequence database at the end of 2023 that includes ~2,800 dogs mapped to the most up-to-date canine reference. This database will allow us to search for rare and unique variants within the Leonbergers to more rapidly identify possible disease causing variants. Due to the very large size of the database, our bioinformatics team is working on coding solutions to allow us to fully utilize this amazing tool.

The University of Bern is also generating a whole genome sequence database mapped to the same most up-to-date canine reference. We will be comparing variants of interest identified between these databases.

Update from Katie Minor

University of Minnesota

Canine Genetics Laboratory

Health Questionnaire: In 2023, the University of Bern – University of Minnesota online Leonberger Health Questionnaire received more than 500 responses in 10 languages. Katie and Anna Letko will be updating the Minnesota and Bern databases to incorporate the responses from these surveys so that the information may be matched with available DNA samples and genetic data. These updates are critical for ongoing research efforts underway, and for projects that the breed group may wish to undertake in the future.

At the University of Bern, Anna will be interrogating their large collection of Leonberger whole genome sequences to search for high impact variants within the breed. These variants for potential associations with traits or medical consequences.

Neonatal encephalopathy: We were contacted by a breeder that had a litter of 4 affected puppies; 1 died suddenly at 2 days of age and the remaining 3 were euthanized at 3 weeks due to severe weakness/limpness and seizure-like episodes. Necropsy of 1 case identified spongy degeneration affecting the cerebellum and cerebellar projections. The whole genome sequencing data has been combined with our SNP array data for mapping, but results were inconclusive. We will next be running the whole genome sequence from 1 of the cases through our pipeline to identify variants that are unique to this case. If this fails, we can also visually inspect potential candidate genes KCNJ10, ATP1B2, ASPA, and ACO2; as well as the mitochondrial DNA.

Update from Katie Minor

University of Minnesota

Canine Genetics Laboratory

Additional genetic causes of Laryngeal Paralysis – Polyneuropathy (LPPN): We believe that the majority of the currently unexplained LPPN diagnoses likely comprise both single gene (monogenic) and multiple gene (polygenic) causes as opposed to acquired diseases (e.g., trauma, infection, inflammation). These as yet unexplained cases have an average age of onset 5.5 years (range 10 weeks – 13 years) and have a higher proportion of males (75%) than females (25%). This same trend is also apparent within our Saint Bernard cohort; they have an average age of onset 3.5 years (range 12 weeks – 10 years), and >80% of cases are males.

Approximately half of Leonberger (and nearly all Saint Bernard) cases have an age of onset of <6 years; younger (non-geriatric) onset forms of disease are more likely to have a more severe form of disease in which a simple genetic cause may be responsible. The close genetic relationship between Leonbergers and Saint Bernards has already proven beneficial to both mapping efforts and validation in the past. The LPN1 and LPPN3 mutation are both also present the Saint Bernards.
Sex distribution of genetically unexplained LPPN Cases (LPN1-NN, LPN2-NN, LPPN3-DN & NN)

	Males	Females	Males %	Females %	Total
Saint Bernard Cases	17	3	85%	15%	20
Leonberger Cases	299	93	76.3%	23.7%	392
Leonberger Controls	258	447	36.6%	63.4%	705

****LHFI support allows us to provide testing to owners with polyneuropathy/laryngeal paralysis affected dogs at no charge. To our knowledge, neither LPN1-DD or LPPN3-DD (homozygous affected) Leonbergers have been born since these tests have been made available to the Leonberger community!**

Update from Katie Minor

University of Minnesota

Canine Genetics Laboratory

Cryptorchidism: During previous studies at the University of Minnesota researching Persistent Müllerian duct syndrome (PMDS), we discovered in the scientific literature that this condition has previously been identified in a 7-year-old Leonberger (Lim CK et al. Vet Radiol Ultrasound, Vol. 56, No. 1, 2015, pp 77–83). PMDS is a rare, recessively inherited, form of male pseudohermaphroditism; they may have remnants of the oviduct, uterus, and cranial part of the vagina. In Miniature Schnauzers with PMDS, ~50% of males are cryptorchid; the external genitalia can otherwise be normal, making it easy to overlook PMDS as the underlying cause.

Recent interrogation of the whole genome sequence database identified a high impact variant in the anti-Müllerian hormone (AMH) gene in a Leonberger. From the 2018 Leonberger Health Survey, 28% of responding Stud Owners have sired puppies that were monorchid/cryptorchid puppies at 8 weeks. Responding Breeders reported 26% of their litters had puppies who were monorchid/cryptorchid at 8 weeks. We would like to genotype Leonbergers with reported cryptorchidism to determine what proportion of cases may possibly be attributable to the AMH variant. This will determine if screening for this variant might help reduce the frequency of cryptorchidism in the breed.

WAS YOUR CRYPTORCHID MALE LEO DIAGNOSED WITH PERSISTENT MÜLLERIAN DUCT SYNDROME (PMDS) WHEN NEUTERED?

If so, please email Katie Minor at the UMN Canine Genetics Laboratory at cgl@umn.edu

Leonberger Sudden Death/Cardiac Arrythmia Study Update

Provided by LHFI Board Member, Susan Manuel
Cardiac Committee Chair & Liason to Lead Researcher Prof. Hannes Lohi (University of Helskinki)

We have exciting preliminary data for the development of an immunoassay as a screening test to identify dogs with high and low risk of arrhythmia. When validated, this could be a helpful blood biomarker for breeding programs and could also motivate dog owners to participate in research.

To validate the immunoassay, we will need to do Holter and echo studies, together with the immunoassay, for selected dogs.

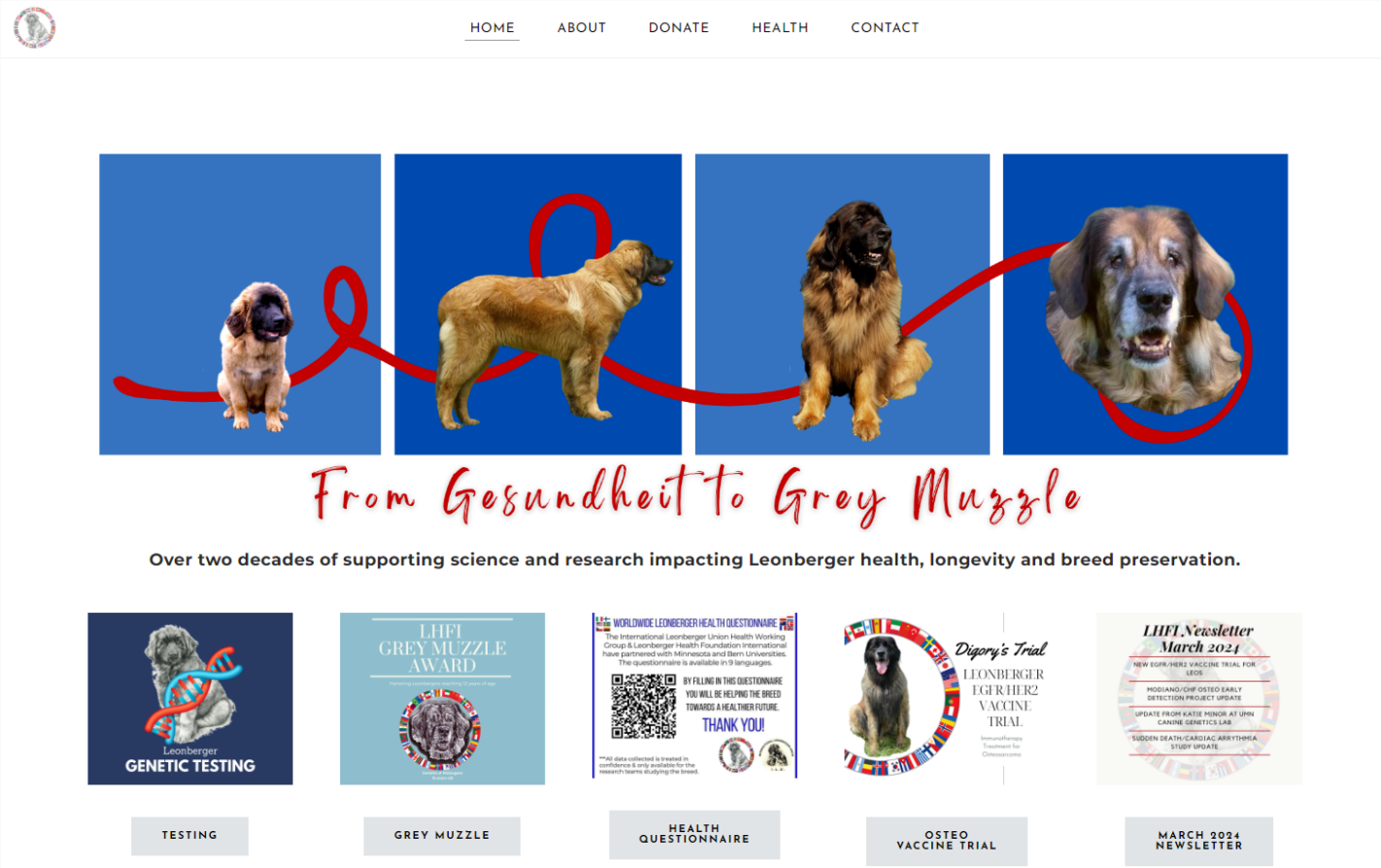
The plan is to set up the immunoassay test in Helsinki for European samples and in Professor Robert Hamilton's lab in Toronto, Canada for US and Canadian samples.

Stay tuned for more information, including how you can participate, in this study.

If you have lost a Leonberger to Sudden Death that is suspected to have been caused by Cardiac Arrythmia, please contact Prof. Lohi at hannes.lohi@helsinki.fi .

VISIT OUR WEBSITE!

WWW.LHFI.ORG



Lots of Information!

- General Health Information of the Leonberger
- Genetic Testing - Why, Where, and How
- Grey Muzzle Award Hall of Honor
- History of the LHFI
- Waltraut Zieher Award
- Meet our mascot, Gesundheit
- Links to Research Publications
- How you and your Leo can participate in research
- Our History of Research Funding
- Ways to Give

THANKYOU

Because of you, the LHFI was able to provide

$60,348.70

to studies directly relevant to Leos in 2023.

$25,107.00 to the University of Minnesota for genetic research in the areas of osteosarcoma, hemangiosarcoma, glaucoma, cardiac diseases, thyroid disease, Addisons Disease, neurological disorders, juvenile renal dysplasia, anal furunculosis, longevity & aging, and population diversity

$2,741.70 to the Worldwide Independent Leonberger Database Foundation (WILD)

$5,000.00 to the University of Helsinki for continued research of genetic basis and biomarkers associated with inherited arrhythmia leading to sudden death in Leonbergers

$27,500.00 to TheraJan LLC to support the Leonberger EGFR Immunotherapy Project for the treatment of Osteosarcoma

Your support is making a genuine and positive impact on the health of our dogs!