RESEARCH PROGRESS REPORT SUMMARY

Grant 02234-MOU: A Novel Approach for Prevention of Canine Hemangiosarcoma

Principal Investigator: Jaime Modiano, VMD, PhD

Research Institution: University of Minnesota

Grant Amount: $432,000.00

Start Date: 3/1/2016 **End Date:** 8/31/2019

Progress Report: Mid-Year 3

Report Due: 8/31/2018 **Report Received:** 8/28/2018

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Original Project Description:

Hemangiosarcoma, an aggressive form of cancer in dogs, is the cause of death for one out of every five Golden Retrievers in the United States. Portuguese Water Dogs and Boxers also have an especially high risk for this disease which is devastating for all dogs. Hemangiosarcoma is incurable partly because the cancer is detected at a very advanced stage when it is resistant to conventional therapies. Thus, an unconventional approach to improve outcomes for hemangiosarcoma patients will involve effective methods for early detection and for disease prevention. This project will pair two novel technologies consisting of a patented test to detect hemangiosarcoma cells in blood samples, and a treatment that attacks the cells that establish and maintain the disease. Three milestones will be met: first, will be to expand understanding of the performance and utility of the blood test for cancer in dogs with active disease; second will be to confirm the utility of the test to predict disease progression in treated dogs. And third will be to establish the performance of the test in the "early detection" setting (dogs at high risk without evidence of active cancer), and thus measure hemangiosarcoma prevention through eradication of the tumor initiating cells with the targeted, investigational drug. This project will create tools to guide further development, licensing and deployment of these paired technologies against cancer, specifically hemangiosarcoma, with an ultimate goal for disease prevention in all dogs.

Funding for the research is provided through the collaborative efforts and generosity of the American Boxer Charitable Foundation, Golden Retriever Foundation, and Portuguese Water Dog Foundation. The AKC Canine Health Foundation supports the funding of this effort and will oversee grant administration and scientific progress reports.

Publications:

Abstracts and Posters

1. DePauw TA, Anderson KL, Graef AJ, Modiano JF. Early Detection for Hemangiosarcoma in Canines. (Poster). UMN Cancer Center GMOC Program Retreat, St. Paul, MN, June 1, 2016
2. DePauw TA, Anderson KL, Graef AJ, Modiano JF. Development of a Test to Detect Hemangiosarcoma Cells in Canine Blood. (Poster). Points of Pride Proceedings (UMN CVM Research Symposium), St. Paul, MN Oct. 5, 2016
3. DePauw TA, Anderson KL, Graef AJ, Modiano JF. Development of a Test to Detect Hemangiosarcoma Cells in Canine Blood (Poster). Masonic Cancer Center Research Symposium, Minneapolis, MN Nov. 2-3, 2016
4. DePauw TA, Anderson KL, Graef AJ, Kim JH, Modiano JF. Early detection and chemoprophylaxis for hemangiosarcoma in dogs (Poster). First Annual University of Minnesota Center for Immunology and University of Chicago Department of Immunology Combined Retreat, Madison, WI, April 11-13, 2017
5. DePauw TA, Anderson KL, Graef AJ, Kim JH, Lewellen MJ, Winter A, Stuebner K, Modiano JF. Development of an Early Detection Test for Hemangiosarcoma in Dogs. (Poster). Points of Pride Proceedings (UMN CVM Research Symposium), St. Paul, MN Oct. 4, 2017. Honorable Mention.

6. DePauw TA, Graef AJ, Dickerson EB, Henson MS, Borgatti A, Vallera DA, Cutter GR, Modiano JF. Circulating Tumor Cell Detection for Hemangiosarcoma Diagnosis. (Abstract and oral presentation). Veterinary Cancer Society Mid-Year meeting, Anchorage, Alaska March 10-13, 2018

7. Park HH, DePauw TA, Modiano JF. Early-Detection of Canine Hemangiosarcoma in Blood Circulation through Adenovirus Transduction. (Poster). College of Veterinary Medicine Comparative Oncology seminar, St. Paul, MN Aug. 9, 2018.

8. DePauw TA, Graef AJ, Modiano JF. (2018). Circulating tumor cell detection for hemangiosarcoma diagnosis in dogs. Submitted to the 2018 Meeting of the Connective Tissue Oncology Society

Lay Publications

9. "Studying Cancer in Dogs as a Path Towards a World Where We No Longer Fear Cancer - A Contemporary Update on Canine Hemangiosarcoma." By Borgatti A, Fahrenkrug AM, Modiano JF. Published in The Alpenhorn (The Official Publication of the Bernese Mountain Dog Club of America), Fall 2017, pp. 64-68
10. "Studying Cancer in Dogs as a Path Towards a World Where We No Longer Fear Cancer - A New Strategy Raises Hope for Hemangiosarcoma." By Borgatti A, Fahrenkrug AM, Modiano JF. Published in The Alpenhorn (The Official Publication of the Bernese Mountain Dog Club of America), Winter 2018

Presentations:

1. DePauw TA, Anderson KL, Graef AG, Modiano JF. Development of a Test to Detect Hemangiosarcoma Cells in Canine Blood. Points of Pride Proceedings (UMN CVM Research Symposium), St. Paul, MN Oct. 5, 2016

2. Modiano JF. Changing the Face of Cancer through Effective Prevention. Featured Speaker for the Golden Retriever National Specialty. October 5, 2016, Placerville, CA

National Professional Presentations

1. "Progress in Treatment and Prevention of Canine Hemangiosarcoma: From the Lab to the Clinic and Back Again." Baker Institute Seminar Series, Cornell University, March 15, 2016, Ithaca, NY
2. "The Role of Companion Animals in Development of Innovative Strategies for Cancer Treatment and Prevention." UC Davis Center for Comparative Medicine Seminar Series, April 5, 2016, Davis, CA
3. "Comparative Approaches to Cancer Research: Deconvoluting our Evolutionary Legacy." Bio-Techne Seminar Series, May 5, 2016, Minneapolis, MN
4. "Swimming Against the Current: A Turning Point in the Battle Against Hemangiosarcoma." Keynote Address, 2016 ICCI Cancer Research Symposium, Ontario Veterinary College, May 17, 2016, Guelph, ON, Canada
5. "Where the Future Lies: Innovations in Cancer Treatment and Prevention." Keynote Address, 3rd World Veterinary Cancer Congress (WVCC), May 25-29 (May 29), 2016, Foz do Iguaçu, Brazil
6. "Clinical and Translational Cancer Research in Companion Animals at the University of Minnesota." Boston Scientific Invited Seminar, Sept. 11, 2017, Arden Hills, MN
7. "Biomarkers, Biotherapeutics, and Other Discoveries at the Boundary of Spontaneous and Experimental Disease in Animals." Annual Meeting of the American College of Veterinary Pathologists, November 7, 2017, Vancouver, BC, Canada
8. "Accelerating to the Future: Companion Animals as Keystones in Innovation for Cancer Prevention and Treatment." Inaugural Penn Vet Cancer Symposium, December 1, 2017, Philadelphia, PA
9. "Advances in Diagnosis and Management of Canine Hemangiosarcoma." State of the Art (SOTA) Presentation (Specialty Symposium), ACVIM Forum, June 13, 2018, Seattle, WA

Local Professional Presentations

10. "Dogs and the Development of Cancer Immuno-Prevention," Pediatric Epidemiology Training Seminar Series, University of Minnesota, August 1, 2016, Minneapolis, MN
11. "Turning the Tables: Using the Hallmarks of Cancer to Develop Effective Strategies for Immunoprevention." Department of Veterinary Clinical Sciences Grand Rounds, University of Minnesota College of Veterinary Medicine, September 15, 2016, St. Paul, MN

12. "Discovery and Applications of Cell-Free Nucleic Acid Markers of Cancer using Comparative Approaches." Masonic Cancer Center, University of Minnesota Seminar Series, January 10, 2017, Minneapolis, MN

Continuing Education and Outreach

13. "Cancer Therapy for Companion Animals." Spring 2016 Mini Medical School, Academic Health Center, University of Minnesota, March 28, 2016, Minneapolis MN
14. "Changing the Face of Cancer through Effective Prevention." Featured Speaker for the Golden Retriever 2016 National Specialty, October 5, 2016, Placerville, CA
15. "Progress in Cancer Treatment and Prevention: an Update from the Animal Cancer Care and Research Program, University of Minnesota." Minnesota Purebred Dog Breeders Association, February 4, 2017, Minneapolis, MN
16. "Canine Cancer and What We Can Do About It." Educational Seminar, Vizsla Club of America 2017 National Specialty, May 4, 2017, Del Mar, CA
17. "Update on Hemangiosarcoma Research." Health Seminar, American Boxer Club, 2017 National Specialty, May 10, 2017, Indianapolis, IN
18. "Canine Cancer and What We Can Do About It." Educational Seminar, National Amateur Retriever Championship, June 16, 2017, Eau Claire, WI
19. "2017 Portuguese Water Dog National Specialty Health Seminar. Hemangiosarcoma Update: Reducing the Impact of Cancer through Effective Prevention." Portuguese Water Dog 2017 National Specialty, September 14, 2017, Warwick, RI
20. "Progress in Cancer Treatment and Prevention: an Update from the Animal Cancer Care and Research Program, University of Minnesota." Norwegian Elkhound Association of America National Specialty Health Seminar, May 9, 2018, Purina Farms, St Louis, MO

Report to Grant Sponsor from Investigator:

During the 30 months that the project has been active, we have made substantial progress toward our objectives, and from the experience we have gained, we have made some adjustments to the experimental set-up. The project goals have not been modified. Specifically:

1. As of August 20, 2018, we had tested the parameters of the detection test on 74 dogs that had diagnoses of hemangiosarcoma, non-malignant spleen masses, other tumors, or no apparent illness.

2. We updated and refined the criteria and the algorithms to identify hemangiosarcoma cells in circulation, and to use this as a test for early detection of hemangiosarcoma for dogs at risk. We have observed cells that have the predicted "hemangiosarcoma progenitor" markers in dogs with other conditions (other tumors such as melanoma, osteosarcoma, and possibly dogs with benign splenic hematomas). So, we have included additional markers to increase confidence in

the results that the cells we can identify in the blood represent hemangiosarcoma progenitors. Even if they do not, their presence seems to be infrequent in healthy dogs from a low-risk population, so we anticipate positive tests will be informative. If the cells of interest are associated with other tumors, it would mean that the test could be deployed more widely, and particularly for other tumors that we expect would be equally responsive to eBAT prevention.

3. We opened Shine On phase-3 on January 2, 2018. As of August 20, 2018, we had enrolled 78 healthy dogs (Golden Retrievers, Boxers, and Portuguese Water Dogs).

Even though we have not completed recruitment to establish sensitivity and specificity for the blood test, we have concluded from our data that the best way to determine if we can use this method to identify dogs with a high probability to eventually develop hemangiosarcoma, is to examine samples from dogs that are presumed to have "moderate risk" (based on age and breed) prospectively, and to follow these dogs through an extended period of time to determine if they eventually develop the disease. To our knowledge, this is the first time this type of bold experiment will have been done in dogs, and we believe the results will be more definitive than what we would obtain by further "tweaking" the assay in the phase-1 and phase-2 design.

By implementing secondary safeguards (additional markers to define "circulating hemangiosarcoma progenitor cells"), we believe that we will be able to protect against false positive results. As part of the modifications to the study, we have been more emphatic in our communication with potential enrollees that, like every clinical trial, Shine On phase-3 is an experiment. Our institutional and programmatic philosophy is to make sure pet owners understand that clinical trials, regardless of their nature, do not have guaranteed benefits, and so, specifically for Shine On, the study website, the enrollment form, the FAQs, and the study reports all include the following statement: "The Shine On study is not intended as a diagnostic for disease. It is an experiment to determine whether the blood test can be used as a tool for early detection of hemangiosarcoma. At this point, we do not know if a negative test result means the dog does not have, or will never get, hemangiosarcoma. We also do not know if a positive test result means that a dog will definitely get hemangiosarcoma. The study is designed to answer some of these questions."

We have added a new parameter to the assay, using a human adenovirus that is "crippled" so it cannot replicate and cause disease, but it can still infect the putative hemangiosarcoma progenitor cells in the blood, but not normal while blood cells. We will continue developing this new parameter to further enhance the potential sensitivity and specificity of the early detection test.

Managing Shine On wholistically, and evaluating all the data in aggregate, will provide more robust data than if we were to parcel out the results by phase. This was not something we could have easily predicted in foresight, but it is clear in hindsight. In other words, since interim analyses are fraught with risk, we have decided to avoid strong conclusions from incomplete data. We will, of course, conduct a complete and thorough analysis of all the data once all the samples are collected and

processed through the assays, and the results, whether positive or negative, will be reported to the AKC CHF and through them to the foundations that have supported the study. We also intend to publish the data in the peer reviewed literature. Regardless of the final result, there is significant innovation in this trial that will be of interest to the biomedical and translational communities. We are excited to document that large-scale trials for early cancer detection are feasible in companion dogs.