



The University of Minnesota Animal Cancer Care and Research Program: Recent News and Progress from the Modiano Lab

Our approach

Our mission is to learn “what makes cancer tick” and to translate that knowledge to better treatments and, ultimately, fewer cases of cancer in animal and human patients. Through innovative research that is relevant and responsive to the needs of the people we serve and their companion animal family members, we are determined to achieve our vision of a world where we need no longer fear cancer.

It serves us well to look back and periodically gauge the progress we have made toward our goal of reducing the cancer burden in animals and people. Our sarcoma program is a particular point of pride; in fact, the Masonic Cancer Center is building one of the premier sarcoma programs in the nation to combat these cancers. Over the past two years, we have maintained a research focus on comparative medicine, emphasizing the roles that genetics and immunology play in the cancer story.

Our recent advances

Soft-tissue sarcomas

Why study sarcoma in dogs? Sarcomas are rare in people, making up only about 1 percent of all human tumors. However, they are relatively common in dogs and especially common in certain breeds. In particular, canine hemangiosarcoma is indistinguishable from a tumor in people called angiosarcoma, both being cancers of cells that form blood vessels. Few cancers are more deadly: the expected survival for dogs with hemangiosarcoma is only four to six months, and most human angiosarcoma patients will succumb to the tumor within three years of diagnosis. The frequency of the canine form of this cancer underscores the need for developing specific treatments to help our companions, but also opens doors to improve our understanding of sarcoma tumors that occur infrequently in humans.

What have we learned from canine hemangiosarcoma? We are especially excited about our progress in understanding this disease. Recently, we have identified regions of the genome that are associated with genetic (heritable) risk for hemangiosarcoma in golden retrievers. This work is part of a large, multi-institutional collaboration that provides a foundation for developing rational strategies for prevention, control, and treatment.

Our team also has shown that the cells that give rise to canine hemangiosarcoma are highly adaptable and can readily change their behavior in response to cues from any environment in which they find themselves. This unique trait contributes to the aggressive, metastatic behavior of this tumor and to its resistance to therapy. The

importance of the interactions between the cells and their environment became even more evident through research in which we showed that, by reducing the tumor's ability to recruit inflammatory cells to its environment, we could inhibit its ability to become established and create clinical disease.

How are we advancing therapy for sarcomas? Our research group showed recently that a compound created at the University of Minnesota specifically destroys hemangiosarcoma “tumor-propagating cells” in the laboratory. This breakthrough provided the impetus to start the SRCBST (“sarc-best”) clinical trial, which seeks to find a safe and effective dose of this innovative drug to treat dogs with hemangiosarcoma and eventually to extend this treatment to people with angiosarcoma and other deadly sarcoma tumors. Concurrently with this trial, we are developing diagnostic tools, such as PET-CT, to better determine the stage and severity of the tumors at the time of diagnosis and to design tests that can predict response to therapy and relapse. The ongoing SRCBST trial began enrolling patients in November 2012, and so far the results are extremely encouraging, showing that this compound not only can be used safely but also appears to improve the survival of dogs with this lethal disease.

Bone sarcomas

Why study bone cancer in dogs? Primary tumors of bone (osteosarcomas) are extremely common in large- and giant-breed dogs. They are the cause of death for up to 15-20 percent of dogs of certain breeds, such as rottweilers and Irish wolfhounds. In humans, osteosarcoma is relatively uncommon, but it is primarily a disease of children and adolescents; about 400 to 600 new cases of osteosarcoma in children are diagnosed each year in the United States. About half of the children diagnosed with this disease will die within 10 years of their diagnosis. Those who survive suffer lifelong effects — from the disease itself, from the aggressive therapy, and from the risk of developing other cancers later in life. Cancer in children also creates a significant psychological burden among patients' families, peers, and community, making the impact of this disease much greater than the number of cases diagnosed.

We can use the clinical similarities between canine and human osteosarcoma, and the relatively frequent occurrence of the disease in dogs, to understand why it happens and how it behaves, which will allow us to develop new therapies for dogs and humans alike. Once we identified specific factors associated with genetic (heritable) risk for this disease in dogs, we began using this information as part of an osteosarcoma research initiative at the College of Veterinary Medicine and the Masonic Cancer Center. This initiative seeks to determine whether genetic risk factors are the same in dogs and humans, identify factors that predict response to therapy, and develop new treatments for this disease that will improve survival and reduce disease- and treatment-related morbidity.

What have we learned from canine osteosarcoma? We have identified molecular abnormalities that drive tumor behavior, and we are currently reviewing the targeted strategies we developed in the laboratory. Through this research, we have developed tests that can predict the clinical behavior of osteosarcoma tumors, potentially allowing clinicians to tailor the type and intensity of therapy to individual patients.

More recently, we have expanded our investigation to understand how osteosarcoma cells communicate with cells in distant sites to improve their metastatic efficiency. We have learned that the primary tumors appear to dispatch “advance scouting parties” consisting of small, tumor-derived vesicles called exosomes, which are loaded with nucleic acids and proteins, to prepare new sites for metastatic growth. These exosomes travel throughout the body in the bloodstream, and as they land in favorable sites, such as the lungs, they further “condition” the tissue to promote the survival of newly arrived tumor cells. Our ongoing efforts seek to characterize the role of osteosarcoma exosomes in the process of lung metastasis and to develop new methods to interfere with this process — delaying or even preventing disease progression and mortality.

How are we advancing therapy for osteosarcoma? We previously developed a novel immunotherapy approach in which we deliver a gene called Fas ligand to the tumor in advance of surgery and chemotherapy. This approach was highly effective in delaying the time to metastasis and increasing survival in approximately half of the treated dogs. We also developed a test to predict which patients were likely respond to this treatment. Current research efforts are aimed at overcoming resistance in the other half of patients. We now have identified a population of supporting cells that inhibits the generation of an immune response against the tumor, and our next steps will be to develop approaches that target and disable these inhibitory cells to reduce tumor resistance to immunotherapy.

We also are in the final stages of evaluating yet another novel immunologic approach to treat bone cancer. In this therapy, developed at the University of Minnesota, *Salmonella* bacteria are genetically altered so they cannot cause disease. They are then used as a guidance system to attack the tumor and incite an immune response. The recently completed clinical trial (OSAL) showed that this therapy could be used safely in dogs with bone cancer; we are currently following up on each dog that participated to determine whether the therapy also will lead to improvements in survival. We hope that our results will help our colleagues at the University of Minnesota Children’s Hospital to accelerate translation of this therapy to children with bone cancer.

Lymphoma and other blood malignancies

Why study lymphoma in dogs? Non-Hodgkin lymphoma is a spectrum of incurable diseases that, unlike other cancers, is increasing in incidence — with rates doubling in the past 30 years. Non-Hodgkin lymphoma accounts for approximately 4 percent of all human tumors; in 2013, there were an estimated 80,000 new cases diagnosed and more than 20,000 deaths from the disease. Approximately one of every 50 Americans alive today will develop non-Hodgkin lymphoma, but we do not know what causes most subtypes of this disease. Even though recent therapeutic gains have been remarkable, they do not extend life for all patients, and cures have been elusive. For example, diffuse large B-cell lymphoma is now a “treatable” cancer, but more than 40 percent of patients with this disease will die within five years of diagnosis. Even more striking is the lack of effective treatments for peripheral T-cell lymphomas; few patients with this disease will survive five years after diagnosis.

The full spectrum of lymphoma tumors is seen in dogs. Not only are these tumors extraordinarily similar to those seen in humans, but they also are more common — an estimated one of every 15 dogs alive today will develop lymphoma. However, this risk is not uniformly distributed among the different breeds; for some breeds, such as golden retrievers, the lifetime risk of developing this disease is much higher. We have demonstrated that the similarities between canine and human lymphomas extend deeply beneath the surface and into the genes that drive these tumors. Furthermore, because the rate at which the disease advances is adapted to the patient's lifespan, we can learn more quickly whether treatments translated from the bench to the bedside are safe and effective in dogs with lymphoma, and eventually develop and apply these for humans as well.

What have we learned from canine lymphoma? We have identified regions of the genome that are associated with genetic (heritable) risk for lymphoma in golden retrievers. This work is part of a large, multi-institutional collaboration that provides a foundation for developing rational strategies for prevention, control, and treatment. Specifically, our research group has developed highly innovative models that allow us to understand how the genetic makeup of these cells influences disease progression and response to therapy. Our efforts to characterize the diversity of lymphomas led us to create a simple diagnostic test that can be used to classify the tumors into three categories based on their behavior. This test, which has been licensed to a commercial partner, will be useful in confirming challenging diagnoses, and it brings us one step closer to tailoring treatment to individual dogs with lymphoma. Even more recently, we have identified a molecular pathway that appears to be essential for tumor survival. We have come up with a method for screening large compound libraries, so we can develop drugs that target and disrupt this pathway and sensitize lymphomas to chemotherapy.

How are we advancing therapy for lymphoma? In humans, monoclonal antibody-based drugs have revolutionized lymphoma treatment, providing relief, life extension, and even cures for this disease. However, the use of antibody-based drugs to treat canine cancer has lagged significantly behind, mostly due to the cost associated with developing reagents that must be specific for each target species. In other words, monoclonal antibodies produced to treat human lymphoma cannot be used to treat dogs with the same disease. But the relative frequency of canine lymphoma, and the public's expectation that their affected canine family members receive the best possible care, spurred several companies to develop antibody reagents intended for diagnostic applications and treatment of dogs with lymphoma. We have partnered with two such companies and have had exclusive access to their compounds. Having these reagents on hand has helped us to develop an active program where we can test how they can be combined with other conventional and immunological treatments, minimizing toxicity and improving quality of life and survival for both canine and human patients with lymphoma.

We firmly believe that immunotherapy for lymphoma will transform the way we treat this disease. But we also realize that it will not be a panacea, and we will need to consider other approaches. With this in mind, we have established more partnerships for testing new, highly targeted drugs for lymphoma therapy. The results from a series of recently completed multi-institutional clinical trials, done in collaboration with our colleagues at

the Ohio State University, has led to the first approval by the Food and Drug Administration (FDA) in over 30 years for a new drug (Verdinexor) to treat newly diagnosed or relapsed canine lymphoma. The quest for newer, better therapeutics continues with other partners.

Cancer immunotherapy

Why use immunotherapy to treat cancer? Immunotherapy is an appealing modality for treating cancer. Cells of the immune system can be made to target and destroy tumors resistant to conventional treatments (such as radiation and chemotherapy) and distant metastasis, the primary cause of death in cancer patients. To aid the immune system in its work, we need to develop strategies that unmask the tumor, which is generally hidden from the immune system. Unmasking the tumor requires breaking up a series of components that make up an immunosuppressive tumor barrier, maximizing the exquisite specificity that we can achieve with immunotherapy while minimizing the potential side effects of immune attack on normal body tissues. The University of Minnesota is a leader in cancer immunotherapy, and we are committed to continuing that tradition.

How are we advancing cancer immunotherapy? We have worked to develop approaches to improve the safety and efficacy of various types of cancer immunotherapy. For example, we showed that injecting a gene encoding a “death molecule” called Fas ligand into tumors changes the profile of inflammation, allowing the immune system to see the tumor and mount a defense against it. In our pivotal clinical study of dogs with bone cancer, this therapy led to a dramatic improvement in survival without toxicity in about half of the patients. Specifically, the fraction of dogs with bone cancer surviving three years was increased from the expected roughly 5 percent with conventional therapy to about 40 percent for dogs in the therapy-responsive group. Current work on this therapy includes testing its efficacy in other cancers and investigating the mechanisms that account for resistance in non-responders.

A similar principle applies to a therapy developed at the University of Minnesota, which uses genetically modified *Salmonella* organisms (crippled so as not to cause disease) as carriers for immune-activating genes. By themselves, the *Salmonella* organisms home to the tumor site and stimulate a strong immune response; adding immune-stimulating genes to the *Salmonella* enhances that response and the likelihood that the response will spread to recognize the tumor. We recently completed enrollment for the OSAL clinical trial showing that this approach can be used safely in dogs, and we are completing follow-up on the enrolled patients to determine whether the therapy also delayed the onset of metastasis and improved survival.

The approaches described above rely on “active immunotherapy,” where the body must generate an immune response against the tumor. We also have been working to develop “passive immunotherapy,” where we provide preformed immunological molecules such as antibodies that attack the tumors. Our main efforts in this area have centered on development of reagents that target a molecule called CD20 that is present on the surface of malignant B-cells (lymphomas). We have worked with two industry partners to study the basic mechanisms that allow such reagents to be used for lymphoma diagnosis and

therapy, intending to optimize their use by providing new options with better outcomes for dogs with lymphoma.

Finally, we are looking for ways to expand applications that use “innate” immunity for cancer therapy. Innate immune cells include macrophages and natural killer cells that are responsible for the earliest stages of an immune response. Our work in this area primarily seeks to determine whether engaging these cells can produce long-term, specific immune responses, a critical step in developing the most effective treatment regimens.

Targeted therapies

Why use targeted therapies to treat cancer? The goal of targeted therapies is to attack the Achilles’ heel of tumors while sparing the normal tissues in the body, in effect increasing the efficacy of therapies and reducing the toxicity. Several approaches have been used to impart specificity to targeted therapies. One approach has been to identify specific proteins that are highly expressed on the tumor surface or in the tumor environment, but less so in normal tissues. A second approach has been to identify protein targets that are uniquely utilized by tumors — ideally those that are essential for the tumor cells but largely irrelevant for normal cells. We have been testing both approaches, assembling teams at the University of Minnesota with the expertise to invent completely new treatments for cancer.

How are we advancing targeted cancer therapies? The first approach has led to what is perhaps the most encouraging trial in our recent arsenal. We first created a drug to target specific receptors that are highly expressed in various cancers and in the blood vessels that feed those cancer cells. The approach to target both receptors in combination created very high specificity for this protein, minimizing the likelihood of collateral damage. Next, the potency and specificity of the drug was confirmed in the laboratory using cells derived from dog sarcomas, and an initial safety profile was obtained that allowed us to bring this drug to canine cancer patients. A clinical trial for dogs with hemangiosarcoma called SRCBST (“sarc-best”) was then designed, using the most contemporary and innovative approaches that allow us to find a safe and effective dose of the drug without having to reach a toxic dose. Finally, the trial was implemented using the infrastructure of the Clinical Investigation Center at the College of Veterinary Medicine. The ongoing SRCBST trial began enrolling patients in November 2012, and so far the results are extremely encouraging, showing that this compound not only can be used safely but also appears to improve the survival of dogs with this lethal disease.

On a parallel track, we used submicroscopic vesicles called nanoparticles, which deliver gene “silencers” to tumor cells. These nanoparticles are so small that approximately 1,000 of them could be lined up along the diameter of a human hair, making it extremely easy for them to penetrate tissues without the need to travel through normal blood vessels. The gene silencer targets a gene that is absolutely essential for the survival of virtually every tumor cell, but is dispensable for most normal cells. Laboratory tests confirmed that this approach would be well-suited to treat a cancer of cats that shares many features of human head and neck cancer. In collaboration with the Minneapolis Veteran’s Administration Hospital, we initiated a clinical trial for cats with oral squamous cell

carcinoma to determine safety and efficacy of both the delivery system and the anticancer gene. We call this trial “Vets Helping Vets Helping Vets” (veterinarians helping veterans helping veterinarians), a win-win-win situation where veterinarians, pets, and humans benefit. Enrollment is ongoing, and we remain on track to meet the trial goals.

Using the second approach, we recently completed a series of multi-institutional clinical trials in collaboration with our colleagues at the Ohio State University. For these trials, we tested a drug called Verdinexor to treat newly diagnosed and relapsed canine lymphomas. This drug inhibits a protein that transports cargo across the nuclear membrane inside the cell. The protein is essential for cancer cells to displace proteins that suppress tumor growth and which must reside inside the nucleus for proper function. Verdinexor inhibits this transport, allowing the tumor suppressors to regain their function and kill tumor cells. The results from these trials were extremely encouraging and led to the first FDA approval of a new drug to treat canine lymphoma in over 30 years.

Finally, we have established a new collaboration with our colleagues in the College of Science and Engineering to launch a completely new approach to diagnose and treat cancer. We will create “nanobots,” minuscule robots that will probe and attack cells from the inside out. The nanobots are internalized by tumor cells and can therefore be used to study cellular activity. The goals of this project are to develop strategies to manipulate the nanobots to distinguish cancer cells from healthy cells, exploiting this specificity such that the nanobots can “shred” tumor cells internally without damaging healthy tissue.

Training and education

A very important part of our mission is training the next generation of veterinarians and scientists. Our philosophy is to select the best and brightest students and provide a stimulating and rigorous environment that encourages innovation, allowing students to develop the skills they will need to succeed in their future careers.

Philanthropic support has been essential for development of training programs at every level: from high school students, undergraduate students, graduate students, and veterinary summer scholars, where students can experience cutting-edge research as part of their education, to our residency program, where we train veterinary specialists in various disciplines, to our fellowship and post-doctoral programs, which are designed to train the next generation of leaders in veterinary cancer research and translational medicine. Former and current trainees in our program include:

- Ms. Katie Anderson, a student in the professional DVM program at the College of Veterinary Medicine, received a fellowship from the Howard Hughes Medical Institute (HHMI) and the Burroughs Wellcome Fund (BWF) in 2013 to study how to improve cancer immunotherapy with Drs. Jaime Modiano at the College of Veterinary Medicine and Dr. Matt Mescher in the Center for Immunology. Ms. Anderson established herself among the elite of students at HHMI, as evidenced by the extension of her fellowship support for a second year. Ms. Anderson’s recent presentation at the meeting of the American Association for Cancer Research drew an enthusiastic response from investigators in academia and industry. After the completion of her HHMI fellowship, Ms.

Anderson will continue working on cancer immunotherapy as a combined degree (DVM/PhD) student at the College of Veterinary Medicine.

- Dr. Claire Cannon joined the College of Veterinary Medicine in 2009 as a resident in veterinary medical oncology. Dr. Cannon completed the credentials for board certification by the American College of Veterinary Internal Medicine in the subspecialty of oncology with distinction in 2012. She then stayed at the University of Minnesota as a post-specialty fellow working with Drs. Modiano and Ahmed through 2013. During her fellowship, Dr. Cannon studied the safety and efficacy of a new, highly innovative treatment for head and neck cancers in cats. This project has the potential to provide an effective therapy not only for cats, but also for people with this severe disease. Dr. Cannon is now an assistant professor of oncology at the University of Tennessee.
- Dr. Daisuke Ito was a postdoc in Dr. Modiano's lab between 2008 and 2012, when he was promoted to the faculty track. The American Veterinary Medical Association and the American Veterinary Medical Foundation recognized Dr. Ito's exceptional performance by awarding him their Young Investigator Award for 2012. He also received a First Award from Morris Animal Foundation to support his transition to faculty. As an assistant professor of oncology and comparative medicine, Dr. Ito now co-leads the Animal Cancer Care and Research comparative lymphoma program, which is a coordinated effort among investigators from the College of Veterinary Medicine, Masonic Cancer Center, Medical School, and College of Pharmacy.
- Ms. Camille McAloney joined our program the summer between her freshman and sophomore years at Augsburg College. Her project was designed to study the role of an enzyme called telomerase in cancer and ageing. Ms. McAloney's work with Dr. Modiano at the College of Veterinary Medicine and Dr. Bagchi at the Masonic Cancer Center continued through the end of her undergraduate career, culminating in a groundbreaking scientific paper. There, the group reported that differences in telomerase date back to the time before dog breeds were established, and therefore are probably not singly responsible for breed-related differences in cancer risk or ageing. Ms. McAloney is now a combined degree (DVM/PhD) student at the College of Veterinary Medicine.
- Ms. Ashley Rodriguez, a 10th grader at the Potomac School in McLean, Virginia, spent the summer of 2013 in the lab, where she applied cutting-edge technology to study the role of S1P in canine hemangiosarcoma. Ms. Rodriguez's results were instrumental for us to obtain a grant from the AKC Canine Health Foundation to continue our important work on this project.
- Dr. Jill Schappa started working in the Modiano lab as a summer scholar at the end of her first year in the professional DVM program. This experience led to a fellowship from the HHMI and BWF to spend a year immersed in research. As the first veterinary student to receive one of these fellowships, Dr. Schappa worked with Dr. Modiano at the College of Veterinary Medicine and Dr. Daniel Vallera at the Masonic Cancer Center to study a new type of therapy for sarcomas. Her work in the lab formed the foundation that allowed us to launch the SRCBST ("sarc-best") clinical trial for canine hemangiosarcoma. Dr.

Schappa earned her doctor of veterinary medicine degree in 2012 and is currently a resident in clinical pathology at the College of Veterinary Medicine. In recognition of her accomplishments, Dr. Schappa was awarded a clinician-scientist fellowship by the AKC Canine Health Foundation in 2013 to study the protective effects of exercise on the bone marrow after treatment with radiation and chemotherapy.

Your role in the process

Please contact Dr. Jaime Modiano at 612-625-7436 or modiano@umn.edu, or Andrea Fahrenkrug at 612-626-6501 or fahre018@umn.edu, if you have any questions about supporting this program. We are excited about the future as we continue to build a bridge to a world where we no longer fear cancer.