PURINA PRO PLAN

DOG Update

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TREATMENT INSIGHTS & DNA TESTING

A Look at Mast Cell Tumor & Juvenile-Onset Laryngeal Paralysis and Polyneuropathy

NEW TREATMENTS FOR MAST CELL TUMOR & DNA TESTING FOR JLPP OFFER HOPE

Advancements in treating canine diseases, such as cancer, come from innovative studies sometimes patterned after progress in human medicine. In this issue of the *Dog Update*, new therapies focused on checkpoint molecules and personalized medicine for mast cell tumor (MCT), the most common skin cancer in dogs, are featured.

Genetic studies of the neurological disease juvenile-onset laryngeal paralysis and polyneuropathy (JLPP) in Rottweilers and Black Russian Terriers show how the autosomal recessive *RAB3GAP1* gene mutation occurred in an ancestral founder long before either breed was formed. The fatal condition affecting puppies can be eliminated in both breeds, thanks to a DNA test.

CANINE MAST CELL TUMOR

A personalized medicine approach to MCTs that are more aggressive because they have a mutation in the *c-kit* gene looked at whether treatment with KIT inhibitors yielded treatment success. Meanwhile, a study of checkpoint molecules is progressing this novel cancer immunotherapy in dogs. The AKC Canine Health Foundation supported both studies.

Douglas Thamm, VMD, DACVIM (Oncology), the Barbara Cox Anthony Professor of Oncology at Colorado State University, led a multi-center study exploring the potential sensitivity of mast cell tumors with *c-kit* gene mutations to KIT-inhibiting drugs. A protein found on the surface of many different types of cells, the KIT protein also may be found in higher than normal amounts or in a changed form on some types of cancer cells, including MCT. The study was published in 2018 in the *Journal of Veterinary Internal Medicine*.

In the clinical trial, Dr. Thamm and his team compared the effectiveness of toceranib (TOC), a KIT inhibitor sold as Palladia™, and vinblastine (VBL), a chemotherapy drug, in

RECENT PUBLISHED RESEARCH FUNDED BY AKC CANINE HEALTH FOUNDATION

The discovery by investigators at Washington State University that Greyhounds and other sighthound breeds have a genetic mutation in the drug metabolizing enzyme (CYP2B11) that may contribute to decreased anesthetic drug metabolism, making them vulnerable to a potentially life-threatening slow recovery from anesthetic drugs, has shed light that more breeds and mixed breeds could be affected.

A report on the findings, published Jan. 9, 2020, in *Nature Scientific Reports*, indicates that as many as one in 50 Golden Retrievers and one in 300 Labrador Retrievers, as well as a handful of other popular breeds, may have the CYP2B11 mutation that could decrease their ability to break down anesthetic drugs.

Greyhounds are among the sighthound breeds with a genetic mutation that causes a slow recovery from anesthetic drugs.

Principal investigator Michael Court, BVSc, PhD, professor and the William R. Jones Endowed Chair, says, "We also suspect that dogs with the mutation may have trouble breaking down drugs other than those used in anesthesia. The challenge now is to provide accurate advice to veterinarians on what drugs and what drug dosages should be used in affected patients."

Lead author of the report is Stephanie Martinez, PhD, a postdoctoral research associate. The researchers are part of the Program in Individualized Medicine (PriMe) at Washington State University College of Veterinary Medicine. The AKC Canine Health Foundation provided funding of $150,000 for phase one of this research from 2016 to 2018 and funding of $172,765 for phase two that began in June 2018 and will finish later this year.

On another front, investigators at North Carolina State University have found a high prevalence of *Bartonella* bacteria in tumors and tissue — but not in blood samples — of dogs with hemangiosarcoma, a cancer of the blood vessels. The study, published Jan. 10, 2020, in *PLOS ONE*, indicates that *Bartonella* could contribute to the cancer yet is difficult to detect because it can hide in the cells lining blood vessel walls.

Hemangiosarcoma is an aggressive, deadly cancer that causes two-thirds of all heart or splenic tumors in dogs and is most common in medium-sized and middle-aged dogs. Hemangiosarcoma is not typically detected until it has reached an advanced stage, resulting in a dismal one-year survival of only 12 to 20 percent.

"Given the established links between chronic inflammation and cancer, we wanted to determine whether chronic infection of blood vessels due to bacteria could be a contributing cause of this cancer," says Edward Breitschwerdt, DVM, DACVIM, the Melanie S. Steele Distinguished Professor of Medicine and Infectious Disease at North Carolina State University College of Veterinary Medicine. "Research in recent years has confirmed that persistent infection or inflammation caused by stealth pathogens is a risk factor for developing cancer later in life."

The AKC Canine Health Foundation provided funding of $219,026 for this study, which began in February 2018 and will continue through January 2021.

treating dogs with MCT with and without *c-kit* gene mutations. "Our hypothesis was that MCT with *c-kit* mutations would have a superior response to toceranib compared to vinblastine," Dr. Thamm says.

Sixty dogs received TOC, of which 20 percent had *c-kit* mutations, and 28 dogs were given VBL, with 30 percent having *c-kit* mutations. The overall response rates were 46 percent for dogs receiving TOC and 30 percent for those on VBL.

"Neither the progression free survival time (length of time during and after treatment that a patient lives without getting worse) nor overall survival time (length of time from the start of treatment that a patient is still alive) was significantly different between the treatment groups," Dr. Thamm says. "As the proportion of dogs with *c-kit* mutations was not different between treatment groups, *c-kit* mutation status did

Purina and the AKC Canine Health Foundation have worked together since 1997 to support canine health research to benefit all dogs.

CANINE CANCER RESEARCH INITIATIVE

Since 1995, the AKC Canine Health Foundation has provided funding of over $12 million to support 207 studies of canine cancer. These investigations have helped scientists learn more about how cancer affects dogs. Discoveries across many types of canine cancer have contributed to earlier diagnoses and more effective treatments and often inform human cancer research via comparative oncology.

"Canine cancer is a devastating disease," says Dr. Diane Brown, CEO of the AKC Canine Health Foundation. "The goal of this research initiative is to provide funds to support research that will advance understanding of the mechanisms underlying cancer and lead to more effective treatments and educational resources for dog owners and veterinarians."

not predict treatment response as we had hoped. Further studies are needed, but this work has helped us to understand the value of these targeted drug therapies."

Another Colorado State University researcher, Steven Dow, DVM, PhD, DACVIM, professor of immunology and director of the Center for Immune and Regenerative Medicine Clinical Sciences, is soon to complete a study of the effectiveness of OX40 checkpoint molecules as a targeted antibody for canine cancer immunotherapy. In human oncology, clinical trials of antibody therapeutics targeting checkpoint molecules, such as PD-1, have shown remarkable success inducing tumor regression and providing cures against a variety of cancers.

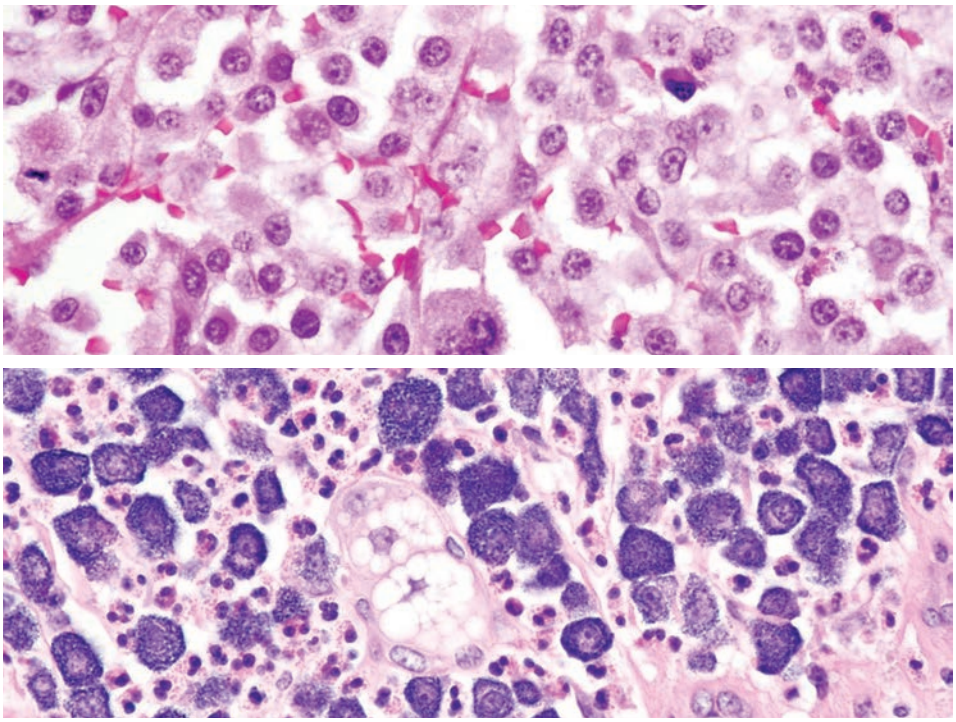
Immune checkpoints regulate the immune system and help to prevent the immune system from attacking cells indiscriminately. Some cancers, however, can protect themselves from attack by stimulating checkpoint targets. Inhibiting checkpoint molecules are targets for cancer immunotherapy due to their potential for use in multiple types of cancer.

"Checkpoint molecules play a key role in regulating T-cell immunity against cancer," Dr. Dow explains. "We are developing a second-generation immunotherapy for dogs that follows the first-generation PD-1 antibodies already underway. In this project, the team is characterizing canine OX40 antibodies to determine how they activate effector T cells in dogs. We want to see if they trigger an immune activation in tumor tissues. There's hope in the near future for completely changing the canine cancer treatment landscape, and that's happening pretty quickly."

The progress being made is promising. MCT can affect any breed of dog or mixed-breed dog. A cancer of a type of white blood cell found throughout the body, mast cells are an important part of the immune system. They contain histamine, heparin and enzymes that break down proteins and help them do their primary function of defending the body against allergens and inflammation. When these cells replicate out of control, they form an aggressive cancer. Besides the skin, MCT can occur in the spleen, liver, gastrointestinal tract, and bone marrow.

When the cancer occurs on the skin, it appears as a red, ulcerated or swollen lump anywhere on the body. A lump may occur suddenly and grow very quickly, or it may be present for many months with the size of the tumor waxing and waning. When the tumor spreads or metastasizes, the most common sites affected are lymph nodes, spleen and liver.

The prognosis for an individual dog with MCT depends on factors such as tumor grade, tumor stage and whether surgery is possible to completely remove the tumor. If MCT is detected early when tumors are small and localized,



The two-tier grading system for MCT developed at Michigan State University separates high-grade aggressive cancers, shown on top, from low-grade tumors, above. The journal* Veterinary Pathology *published an article on the two-tier grading system in 2011.

treatment success is more likely. On the other hand, if the tumor has spread beyond the lymph nodes and is located in areas other than the skin, the prognosis is generally poor.

A two-tier grading system for MCT, developed in 2011 by researchers at Michigan State University, has helped to distinguish aggressive tumors from those likely to respond to treatment. Prior to this, a dog's prognosis and therapy were based on a tumor's histologic grade, or the degree of abnormality of cancer cells as seen per microscopic evaluation. The three-tier Patnaik grading system commonly used by veterinary pathologists is more subjective and thus more open to interpretation variability among pathologists of the same tumor sample. This has caused questionable prognostic value of histologic grading.

The two-tier grading system provides a uniform method for classifying tumors. "The idea of our grading system was to split out dogs with highly aggressive tumors from those with low-grade tumors," says Matti Kiupel, DVM, PhD, DACVP, professor of anatomic pathology at Michigan State University. "The goal was to ensure that dogs did not receive unnecessary therapy.

"Our grading system classifies high-grade MCT as those having a significantly shorter time to metastasis or new tumor development with shorter survival time. In our study, the median survival time was less than four months for high-grade MCTs but more than two years for low-grade MCTs."

Evaluating whether spay and neuter surgeries increase the risk of disease, including MCT, investigators at the University of California-Davis studied 12 dog breeds. Led by Benjamin Hart, DVM, PhD, Distinguished Professor Emeritus, the study, which was funded by the AKC Canine Health Foundation, found that in medium and large breeds, early spay and neuter surgeries before 1 year of age were associated with a higher incidence of several common cancers, with MCT among them.

"There's hope in the near future for completely changing the canine cancer treatment landscape, and that's happening pretty quickly."

Steven Dow, DVM, PhD, DACVIM, professor of immunology and director of the Center for Immune and Regenerative Medicine Clinical Sciences at Colorado State University

Rottweilers and Black Russian Terriers carry the exact same mutation for juvenile-onset laryngeal paralysis and polyneuropathy, indicating that the mutation likely occurred in a dog ancestral to the formation of both breeds.

JLPP ANCESTRAL FOUNDERS

A progressive, fatal neurological condition, juvenile-onset laryngeal paralysis and polyneuropathy (JLPP) is heartbreaking for those whose dogs inherit the disease. Recognized in the late 1990s when veterinary neurologists in the U.S. and Europe began seeing Rottweiler puppies around 3 to 8 months of age with signs of JLPP, the disease was not identified. They suspected it was a hereditary condition because the clinical signs of laryngeal paralysis and cataracts are more common in older dogs.

Affected puppies were clumsy and had difficulty climbing stairs. The condition affected all their legs, though the rear legs were more involved. Breathing difficulties were common, especially with exercise and excitement. In all the dogs, the severity of disease progressed quickly, and the puppies were euthanized by 17 weeks of age. Although the cause was not known, it was apparent that this disease was different than other neurological conditions in young Rottweilers.

Nearly 20 years later, an autosomal recessive gene mutation was discovered for a similar juvenile-onset disease in Black Russian Terriers. "Once we had the mutation in Black Russian Terriers, we were able to test Rottweilers to see if it was the same mutation," says Dennis O'Brien, DVM, PhD, the Chancellor's Chair in Comparative Neurology at the University of Missouri College of Veterinary Medicine, who worked on the gene mutation in both breeds. "It proved to be the same mutation."

Affected dogs inherit copies of the *RAB3GAP1* gene mutation from their sire and dam, and carrier dogs are heterozygous or have only one copy of the *RAB3GAP1* gene mutation. A deletion in the *RAB3GAP1* gene affects a protein involved in

membrane trafficking and causes JLPP in Rottweilers and Black Russian Terriers. The discovery of the mutation allowed for the development of a DNA test to aid in diagnosing JLPP and identifying carriers.

"It is likely the same founder mutation event was the source of the *RAB3GAP1* gene mutation in both breeds," Dr. O'Brien says. "When Stalin (Soviet Communist leader Joseph Stalin) wanted to create a guard dog capable of standing Russian winters in the 1930s, a heavy-coated Russian breed was crossed with Standard Schnauzers and Rottweilers to produce the Black Russian Terrier. The mutation was probably in Rottweilers, then got passed on to Black Russian Terriers."

A disease that occurs in young Rottweilers and Black Russian Terriers, JLPP affects neurological signaling that travels from the brain through nerves. In puppies born with JLPP, the vagus nerve, the longest and most complex cranial nerve, fails to develop normally. In a normal dog, the vagus nerve signals the muscles of the voice box or larynx, creating the sound of a bark when the vocal folds vibrate as air moves over them and assisting breathing by pulling the vocal folds aside so air can move easily into the lungs. The vagus nerve also helps to close the larynx when the dog swallows to prevent choking on food or water.

When the nerves are unable to convey messages properly, the muscles become weak or paralyzed. The vagus nerve is often affected first, explaining why laryngeal paralysis is generally the first sign of JLPP. The vocal folds cannot be pulled out of the way as the dog breathes. Instead, they vibrate noisily and obstruct the flow of air into the lungs. When a dog regurgitates food or water, this can result in aspiration pneumonia.

The second longest nerve in the body, the sciatic nerve, is typically affected next. This accounts for why dogs have difficulty getting up and wobble as they walk. Usually the rear legs are affected first, followed by the front legs.

Treatment for JLPP is limited. The breathing problems caused by laryngeal paralysis can be improved with surgery, but the progressive disease eventually wins over. Weakness and coordination problems become more severe, and dogs can develop problems swallowing. While a rare condition, eventually all dogs have to be euthanized.

Genetic testing of breeding stock combined with selective breeding provide the best approaches to ensure that two JLPP carriers are never bred together and no affected puppies are produced. The Orthopedic Foundation for Animals (OFA) provides information on its website about ordering the JLPP test that is processed at the University of Missouri Small Animal Molecular Genetics Lab.

"Quality carriers can be bred to non-carrier dogs and then you would replace the carrier parent with a quality non-carrier offspring," says Jerold Bell, DVM, adjunct professor of genetics at Cummings School of Veterinary Medicine at Tufts University and a genetic advisor to national parent breed clubs. "In this way, breeding lines — and breed genetic diversity — are not abandoned, and testable disease liability genes can be lost in one generation.

Breeders who produce affected dogs are advised not to eliminate the breeding of carriers, as this could narrow the gene pool and reduce desirable genetic diversity in the breed. "Narrowing the gene pool by not breeding carriers may inadvertently increase the incidence of other hereditary problems lurking in the bloodlines of Rottweilers and Black Russian Terriers thatare free of the JLPP mutation," Dr. Bell says. ■

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