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ROTTWEILER Update

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OSTEOSARCOMA RESEARCH

A New Vaccine & Gene Expression Profiling

ROTTWEILERS MAY BENEFIT FROM ADVANCES IN OSTEOSARCOMA RESEARCH

A tough, complicated cancer, no one knows exactly what causes osteosarcoma, and efforts to better understand the cancer have been challenging.

“Benelli,” or “Benny” for short, was 5 years old in 2007 when he was diagnosed with osteosarcoma in his right front leg. The gregarious Rottweiler, titled in conformation, obedience, carting, and as a therapy dog, seemed to have a bum leg from the start. At 18 months old, Benny, painfully lame, had surgery for elbow dysplasia.

When the lameness came back three years later, owners Kelly and Dr. Anthony Siptunas of Wrightsville, Pennsylvania, took Benny to be examined at Cornell University Com-

panion Animal Hospital. Rottweiler owners since 1988, they adored the intelligence and versatility of the working breed. Benny, one of four male Rotties who shared their home, had a special gift as a certified reading education assistance dog.

“We wanted to know what was going on with Benny’s leg,” says Kelly Siptunas. “Benny was unbelievably smart and intuitive. Everyone loved him. He loved all people, especially children.”

Initially diagnosed with severe osteoarthritis in his leg and elbow, Benny, they learned a few months later, also had early-stage osteosarcoma, a tiny lesion invisible on a radiograph but apparent on a bone scan. Their options were dismal.

A tough, complicated cancer, no one knows exactly what causes osteosarcoma. Standard treatment for the aggressive, most common bone cancer in dogs is amputation of the affected leg followed by chemotherapy to help reduce the risk of metastasis. About 50 percent of dogs receiving the standard treatment survive nine to 11 months after diagnosis and surgery, about 20 percent survive two years, and about 5 percent survive three years. When dogs are treated with amputation alone, the median survival is six to seven months. More than 90 percent of dogs receiving no treatment or alternative holistic treatments die from metastatic disease within three to five months.



Kelly Siptunas and “Benny” (AM/CAN CH Oakview’s Roughrider v Esmond RN, CI, TT, CGC, RTD, TDI, R.E.A.D certified), wearing a brace on his front leg treated for osteosarcoma, receive the American Kennel Club’s ACE (Award for Canine Excellence) Award for Pet Therapy in 2012.



A certified reading education assistance dog, Benny was adored by children because he listened attentively and patiently as they practiced reading aloud.

Efforts to better understand osteosarcoma have been challenging. Recent advances include a new canine osteosarcoma vaccine developed at the University of Pennsylvania that shows promise in extending survival following surgery and chemotherapy. At the University of Minnesota, investigators are using gene expression profiling to help them understand the biological behavior of osteosarcoma tumors. Insights may help them learn how quickly the disease progresses locally, how quickly it spreads to other sites, and what factors may accelerate or delay disease progression.

Meanwhile, the surgery team at Cornell evaluated Benny’s case, and determined he was an ideal candidate for a limb-sparing surgery known as longitudinal bone transport. The procedure removes the affected portion of the bone and then forms new bone, or a free transport segment, via osteotomy, eventually fusing the defective area with healthy bone.

“We didn’t want to amputate his leg, he was so young,” Kelly Skiptunas says.

Post-surgical care involved Benny wearing an external fixator daily for two months. Every four hours, the family adjusted the fixator to help stretch the bone so it would grow, and every night, Dr. Skiptunas unwrapped the bandages and carefully cleaned the surgical site to reduce the risk of infection. Benny breezed through chemotherapy with no side effects and continued his important job at read-to-a-dog programs, where children gathered around him to take their turns reading aloud. He and Kelly Skiptunas also visited hospitals, rehabilitation centers and assisted-living facilities.

When he was 10 years old in 2012, Benny became the first Rottweiler to receive the American Kennel Club’s ACE (Award for Canine Excellence) Award for Pet Therapy honoring his many contributions as a therapy and reading education assistance

THINGS TO KNOW ABOUT CANINE OSTEOSARCOMA

- It is the most common bone cancer in dogs
- It can occur in any bone, but the legs account for 75 to 85 percent of cases
- Virtually all dogs from large and giant breeds, as well as large and giant mixed-breed dogs, are at greater risk for this disease
- Signs include lameness, swelling at the tumor site and pain
- Irritability, aggression, loss of appetite, weight loss, whimpering, sleeplessness, and reluctance to exercise often relate to pain
- Standard treatment involves amputation of the affected leg and chemotherapy

Source: The National Canine Cancer Foundation

“The *Listeria* vaccine essentially wakes us the immune system and directs it to specifically eliminate the remaining tumor cells by delivering a target tumor molecule into the system.”

Nicola Mason, BVetMed, PhD, DACVIM, associate professor of medicine and pathobiology, University of Pennsylvania

dog. It was a proud day when he and Kelly Skiptunas received the honor at the AKC National Championship. Three months later — five years after being diagnosed with osteosarcoma — Benny passed away from another, unrelated cancer, hemangiosarcoma.

A PROMISING VACCINE

Greater understanding about how tumors and the immune system interact have contributed to the development of a promising osteosarcoma vaccine immunotherapy, says Nicola Mason, BVetMed, PhD, DACVIM, associate professor of medicine and pathobiology at the University of Pennsylvania.

A pilot study of 18 dogs treated with a live recombinant, HER2-expressing *Listeria* (ADXS31-164) vaccine showed it doubled the survival rate compared to an historical control group. The dogs in the study had surgery to remove their primary tumor followed by four chemotherapy treatments, and then they received three doses of the vaccine every three weeks. The median survival was 956 days compared to 423 days for the control group.

Funded by Advaxis Inc., a human biotechnology company, the phase-one study conducted at the University of Pennsylvania included two Rottweilers. “One was a 7-year-old male and the other was a 6 1/2-year-old bitch,” says Dr. Mason. “Both had forelimb lesions and underwent amputation, follow-up chemotherapy and then received the vaccine. Both dogs did very well; the male lived 956 days following the vaccine treatment, and the female lived 886 days.”

The vaccine uses a genetically modified *Listeria* to deliver a tumor-associated antigen to stimulate the immune system and activate cytotoxic (killer) T cells. These killer T cells specifically recognize cancer

cells that remain in the body after standard of care treatment and eliminate them, thus delaying or preventing relapse.

Listeria monocytogenes is a bacterium that triggers a strong cellular immune response. Although in people unmodified *Listeria* can cause a serious and sometimes deadly infection when contaminated food is ingested, the bacteria in this vaccine has been genetically attenuated, or weakened, so as not to cause infection or disease.

Explaining the rationale behind the vaccine, Dr. Mason says, “most dogs relapse with metastatic disease after chemotherapy, suggesting that not all cancer cells are eliminated by chemotherapy. The *Listeria* vaccine essentially wakes up the immune system and directs it to specifically eliminate remaining tumor cells by delivering a target tumor molecule into the system.

“Immune cells that have been educated by the vaccine will then seek out cells that express the target molecule and specifically kill them. Essentially, we are aiming to generate an effective anti-tumor immune response.”

A phase-two clinical trial, sponsored by Morris Animal Foundation, has begun at 11 university hospitals across the U.S. and Canada using the ADXS31-164 vaccine. The study, performed through the Comparative Oncology Trials Consortium, aims to treat 80 dogs with the vaccine following standard of care amputation and chemotherapy and determine whether these promising results are also seen in a larger group of dogs. The trial also aims to evaluate immune parameters that might predict “responders” versus “non-responders.”

Aratana Therapeutics, a company that markets innovative therapies for dogs and cats, has received a conditional license for a lyophilized, or freeze-dried, version of this vaccine.

The company hopes to receive full licensure in the next year.

To be eligible to participate in the clinical trial, dogs cannot have evidence of metastatic disease at the time of amputation surgery. If dogs develop metastatic disease during their chemotherapy and before receiving the vaccine, owners have the option of fast-tracking their dog to receive the vaccine.

“About one-third of dogs develop metastasis before their fourth chemotherapy treatment,” says Dr. Mason. “By including the fast-track group in this study, we can determine whether this approach can delay the progression of grossly evident metastatic disease.”

In addition to improving the standard of care for dogs with osteosarcoma, the long-term goal of the University of Pennsylvania researchers is to learn whether the same vaccine could be used to help children with bone cancer. In humans, the majority of osteosarcoma cases occur in children.

“Taking a comparative oncology approach, we believe that our canine patients may help to guide a pediatric human clinical trial so we can learn whether this approach might benefit both species,” says Dr. Mason.

A POTENTIAL CANCER DIAGNOSTIC TEST

Cancer researchers at the University of Minnesota are working to identify the molecular properties of various tumor types, including osteosarcoma, in an effort to gather information that will help them diagnose cancer risk and predict disease behavior more accurately. Their ultimate goal is to develop effective strategies to treat and eventually prevent osteosarcoma in both dogs and children.

A recent University of Minnesota osteosarcoma study, published in the January 2018 issue of *Cancer Research*, reported on evaluating

ROTTWEILERS WITH OSTEOSARCOMA MAY BE CANDIDATES FOR VIGOR TRIAL

[A novel oncolytic immunotherapy for treatment of canine osteosarcoma is being investigated at the University of Minnesota.](#) The study is designed to learn more about how well a genetically modified vesicular stomatitis virus (VSV) kills cancer cells.

Dogs chosen for the study will receive either the VSV treatment or a placebo. Neither the owner nor the investigators will know which treatment the dogs are given. Owners and dogs will benefit because the participants will receive the best available standard of care for this disease, including amputation of the affected leg and chemotherapy. Additionally, advanced imaging studies will allow veterinarians caring for the dogs to determine disease progression at an earlier time when other treatment alternatives still can be considered.

To participate, owners must cover the cost of a screening appointment and diagnostics to determine if their dog is a good candidate for the study. Once accepted, owners must be able to take their dogs for periodic recheck visits. The study covers up to \$8,600 in care, which includes the VSV treatment, surgery and part of the costs for chemotherapy.

To be eligible, dogs must:

- Be diagnosed with osteosarcoma in a limb bone
- Weigh more than 44 pounds
- Be healthy, with no significant health concerns
- Be spayed or neutered
- Be current on vaccinations, flea and tick prevention, and heartworm protection
- Have no evidence of metastasis on a chest X-ray
- Have had no previous treatment for their tumor

For information about participating in the study, please contact Andrea Eckert, research study technician, at 612-625-3157 or aleckert@umn.edu.



“While the GCESS method was developed using osteosarcoma as a model, we found it is widely applicable to virtually any type of tumor where genome-wide sequencing information can be obtained.”

Jaime Modiano, VMD, PhD, the Perlman Endowed Chair of animal oncology, University of Minnesota

the transcriptional profile of osteosarcoma tumors in dogs, humans and mice.¹ Also known as gene expression profiling, a transcriptional profile quantifies the abundance of messenger RNA (mRNA) molecules for every gene in cells or tissues.

RNA, which is short for ribonucleic acid, acts as a messenger in cells, transcribing genetic information from DNA into amino-acid sequences that become the protein products of gene expression. The differential patterns of gene expression in tumors from individual patients allow researchers to categorize cancers, such as osteosarcoma, into subgroups with different behaviors and different prognoses.

The comparative medicine approach used by the University of Minnesota researchers, gleaning information from dogs, mice and humans, provides opportunities to better understand osteosarcoma. The study team was made up of scientists with diverse backgrounds, including bioinformatics, genetics and genomics, pathology, immunology, molecular biology, cancer biology, and stem-cell biology.

One of the study investigators, Jaime Modiano, VMD, PhD, the Perlman Endowed Chair of animal oncology at the University of Minnesota, says, “Human, dog and mouse osteosarcoma tumors share certain clinical and molecular similarities, but they also have important species-specific differences. Insights gained from each species help us improve our overall understanding of the disease.”

[A rare cancer in people, with 400 to 600 new cases diagnosed annually in the U.S.](#), osteosarcoma is much more common in dogs. The small number of human tumors available for study compounds the ability of researchers to study the rare but deadly cancer. In children, adolescents and young adults, the

five-year survival for osteosarcoma is around 80 percent, and the 10-year survival is 50 to 60 percent.

“One of our goals has been to define peculiar genetic traits that may contribute to osteosarcoma in dogs and whether any of these traits may also contribute to this disease in people,” Dr. Modiano explains. “Our present goal is to understand how specific mutations help tumors mold their environment and evade the immune system. Identifying such mutations, in dogs or humans, will help us develop tools to diagnose the disease earlier when interventions are more likely to produce long-term benefits and to design effective strategies that are better tailored to each individual patient.”

The researchers used next-generation sequencing to analyze all of the mRNA in human, dog and mouse tumors. “Our hypothesis was that despite the highly complex karyotypes (chromosomal variation) seen in osteosarcoma, transcriptional profiles would allow us to categorize this cancer into distinctive, clinically relevant subtypes,” says Dr. Modiano.

Samples from newly diagnosed human and dog osteosarcoma patients that had not yet received any therapy were included in the study. The dogs’ samples came from a large number of breeds, including many Rottweilers and Golden Retrievers, obtained between 1999 and 2012.

The most exciting development from the study was the creation of the gene cluster expression summary score (GCESS) methodology to categorize tumors. The GCESS captures information from many genes that are turned on and off coordinately, likely by the same mechanisms, says Dr. Modiano.

“Because of the sheer size of the clusters and their tight correlation, the GCESS method is significantly

more powerful to achieve this classification than biomarkers that use a single or even a few genes or molecules,” he says. “While the GCESS method was developed using osteosarcoma as a model, we found it is widely applicable to virtually any type of tumor where genome-wide sequencing information can be obtained.”

Using the GCESS method, the research team identified three clusters that were conserved across all three species and that had clinical significance in at least one species. One cluster, discovered by the team as part of an earlier study, was strongly associated with genes that control cell division and DNA repair. The investigators confirmed their previous observation that the relative expression of this cluster was highly predictive for overall survival in dogs with osteosarcoma independent of treatment.

The other two clusters were indicative of immune cells being present within the tumor. “Interestingly, the relative abundance of immune-cell transcripts was a strong predictor for outcome in human patients, but not in dogs with osteosarcoma,” says Dr. Modiano. “The reasons for this are unclear, but they provide a stark illustration of species differences that must be considered when dogs are used as models for human cancer causation or cancer treatments. Most importantly, these differences do not negate the utility of comparative approaches. To the contrary, understanding these differences makes it possible to design better experiments and to obtain more robust answers.”

Reflecting on her experience with Benny’s osteosarcoma, Kelly



A conformation champion, Benny also was active in obedience, carting, and working as a therapy dog.

Skiptunas says, “The surgery doubled his life span. Now, looking back at what we went through, I don’t know if we could do it again. I am so thankful for the extra years we had with him. He was special to us and to so many other people through his gentle, unassuming nature as a therapy dog.” ■

¹ Scott MC, Temiz NA, Sarver AE, et al. Comparative Transcriptome Analysis Quantifies Immune-Cell Transcript Levels, Metastatic Progression and Survival in Osteosarcoma. *Cancer Research*. 2018; 78(2):326-337.

Purina thanks Roberta Kelley-Martin, president of the Rottweiler Health Foundation, for helping us to identify this topic for the *Rottweiler Update*.

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RECORD YEAR FOR NATIONAL SPECIALTIES AT PURINA FARMS

A record 38 National Specialties will be held this year at Purina Farms. The versatility of the Best in Class show and trial venue combined with its prime location in the middle of the country appeal to parent clubs, with many returning year after year. Custom-built for the dog fancy, the Purina Event Center offers a spacious indoor exhibition space complete with a separate benching and grooming area, dog bathing room, conference rooms, banquet rooms, Checkerboard Café, and numerous other amenities. The 346-acre property includes facilities for herding, tracking, coursing, diving dog, earthdog, and many more activities. Purina Farms is in Gray Summit, Missouri, about one hour from St. Louis.



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PURINA PRO PLAN INTRODUCES SAVOR WITH PROBIOTICS FOR DOGS

Purina Pro Plan is introducing *SAVOR* formulas for puppies and adult dogs made with guaranteed live probiotics to support digestive health. *SAVOR* Shredded Blend Chicken & Rice Puppy Formula, made with chicken as the No. 1 ingredient, contains DHA from omega-rich fish oil to help nourish brain and vision development and is rich in antioxidants to help support a puppy's developing immune system. The complete and balanced puppy food also has calcium, phosphorus and other minerals to help build strong teeth and bones.

The adult dog foods now with probiotics are: *SAVOR* Shredded Blend Small Breed Chicken & Rice Formula and *SAVOR* Shredded Blend Large Breed. This small-breed food is high in protein, including chicken as the No. 1 ingredient, to meet the needs of highly active

small dogs, as well as calcium, phosphorus and other minerals to help maintain strong teeth and bones.

Vitamin A and linoleic acid, an omega-6 fatty acid, nourish healthy skin and coat.

The large-breed formula contains high-quality protein, including chicken as the No. 1 ingredient, plus EPA and glucosamine to help support joint health and mobility. Optimal protein and fat levels help maintain lean muscle and ideal weight and body condition.

Both of these adult formulas also have a natural prebiotic fiber, sourced from wheat bran, to help support digestive health. *Purina Pro Plan* Shredded Blend formulas feature a crunchy kibble combined with tender, shredded pieces for delicious taste and texture.

Look for probiotics coming to other *SAVOR* formulas later this year.



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DEADLINE IS JULY 31 FOR PRO CLUB MEMBERS TO SUBMIT WEIGHT CIRCLES

Don't miss the cutoff of July 31, 2018, for mailing in weight circles. After this date, weight circles will no longer be accepted, as *Purina Pro Club* is introducing a new receipt submission program that will allow you to get your Purina Points into your account faster by taking a picture of your receipt from purchases of eligible *Purina* dog food on your smartphone or scanning the receipt into your computer and submitting it online. Your Purina Points will show up in your account by the end of the day in most cases if

uploaded correctly. You can start submitting receipts now, though once you submit receipts, you will not be able to mail in any more weight circles. To get started, click on the link below that will take you to helpful tools — instructions and a video — and then log on to your *Pro Club* account, register and begin submitting receipts.

[GO TO HELPFUL TOOLS](#)

