

PERSPECTIVES

by Rhonda Hovan

Health Seminar at the National Specialty

The GRCA Health Seminar at the 2000 National Specialty featured canine cancer researchers Jaime Modiano, VMD, PhD, and Stuart Helfand, DVM, as the speakers. Prior to the National, GRCA had received some requests to offer the seminar on either video or audio tape, and did investigate those options. Due to a number of considerations (cost, quality, feasibility), a decision was made to transcribe the seminar instead, and then make the transcription available via the GRCA Web site. Barb Rollins generously volunteered to prepare the transcription.

After many weeks of difficult and diligent work, Barb completed the transcription — an amazing 35 single-spaced, typed pages! I don't think anyone involved realized in advance what an undertaking this would be. And in fact, the presenters themselves were startled at the massive amount of editing that would have been required for them to turn their spoken-word transcript into a readable document. Unfortunately, it would have required a time commitment that they were simply not able to offer.

While this amount of technical reading may have been tedious and overwhelming in research detail for even the most dedicated scientific reader, there was also a more serious concern. Since it was presented at the National with slides, gestures, and all of the voice inflections that accompany a live talk, it simply did not come across as coherently in written form — and in some instances, meanings were distorted.

Therefore, in a mutual decision between the doctors and the Board to permit as many members as possible to benefit, we decided to prepare a summary of the seminar. As one of the doctors noted, this was a learning experience for all of us. We hope that the GRCA membership will understand that all involved are doing their best to share as much as possible of the seminar with those who were unable to attend.

Below you will find the summary, which approximately follows the format of the seminar, in which the doctors alternated speaking. Although with some overlap, Dr. Modiano spoke primarily from the perspective of a research pathologist, while Dr. Helfand represented the perspective of a clinical oncologist and researcher.

Introduction (Modiano)

For most of recorded history, cancer has been a death sentence. Treatments were crude and experimental, and included attempts to cut them out, burn them out, or put potions on them. About 100 years ago, advances in surgery and anesthesia led to somewhat improved treatment options. As scientists began to understand more about the processes of uncontrolled cell division, cancer treatments developed to include chemotherapy and radiation.

In the 1970s the war on cancer brought about significant advances. Researchers worked to understand cancer on a cellular level, and treatments continued to improve. Some cancers became curable, and more was understood about reducing cancer risks.

More recent research has also focused on understanding cancer on a genetic and molecular level — not just how cells behave, but what directs them to behave that way. Understanding the genetic and molecular mechanisms of cancers will permit scientists to develop specifically targeted treatments called *rational treatment strategies*, as well as rational prevention strategies.

Nature and Nurture (Modiano)

Researchers must attempt to unravel the complex puzzle of how and which heritable factors and environmental factors impact the relative risk of cancer, both separately and interactively. One way of viewing cancer is as a Russian roulette, in which the heritable component of cancer conveys a certain degree of risk, and then the environment modifies that risk. There is no way to know for sure whether cancer will occur, but the higher the combined risk of genetics and environment, the higher the odds that cancer will develop.

One important concept to understand about cancer is that it is a genetic disease; that is, the disease occurs because of errors at the genetic level. But it is also important to understand that “genetic” does not necessarily mean heritable. If an error originates in somatic cells (body cells not passed to the next generation), then that particular error will not be inherited. If the error originates in germ cells (sperm

and ova), then the error will be passed on to the next generation. (For a more detailed explanation of genetic errors, please see *Perspectives* in the May-June '00 *GRNews*.)



Jaime Modiano, VMD, PhD begins the seminar with an explanation of the current research being undertaken.

Two types of genes in which errors (mutations) may lead to cancer are *oncogenes* and *tumor suppressor genes*. Oncogenes function to promote cell growth, while tumor suppressor genes function to suppress cell growth. Thus, mutations in these genes which cause them to malfunction may lead to unregulated cell division, or cells which do not die when they are supposed to (“programmed cell death,” or *apoptosis*). When such mutations occur in somatic cells, they are not heritable, and therefore would not be under the influence of breeding decisions.

But mutations may also occur in a dog’s germ cells, which are then passed to its puppies. Since genes are inherited in pairs — one from each parent — a puppy may inherit a mutated, nonfunctional copy of an oncogene or tumor suppressor gene from one parent and a normal copy of the same gene from the other parent. Such a pup can function normally with the one good copy of the gene. (Pups who inherit nonfunctional copies of the same gene from both parents typically do not survive.) But this heterozygous (normal/abnormal pair) gene will exist in every cell of the pup’s body, and is susceptible to further mutation throughout its lifetime. If by random chance or as a result of a high-risk environment a mutation occurs which knocks out the functional copy of this gene in a cell, that cell will no longer have the ability to regulate its growth. This is called *loss of heterozygosity*, and can be the beginning of a cancer.

It should be clear from this description that a dog who is heterozygous for an important oncogene or tumor suppressor gene will be at significantly increased risk for cancer. That is, while mutations are a regular occurrence during cell division, it is far less likely that a dog who has two normal copies of a gene will have mutations in both copies than it is that a single mutation will occur in the only good gene of a heterozygous dog. And this is borne out with what is known about certain genes. In an extreme example, a gene called *mts-1* (multiple tumor suppressor 1) can have a mutant copy

of a protein called p16. When there is a mutant copy of p16 in people, their risk of some cancers is nearly 1,000 times greater than in the general population. And when the mutation exists in mice, more than 90 percent will have cancer by six months old (young adulthood).

There are a few dozen tumor suppressor genes that are under investigation. Some important ones that breeders will probably hear more about in coming years are *p53*, *mts-1*, *PTEN*, *RB-1*, and *waf-1*.

Clinical Presentation and Diagnosis of Lymphoma (Helfand)

Dogs with lymphoma presented to a veterinarian for evaluation typically fall into one of two categories: either they have lumps and are not sick, or they have lumps and are sick. The lumps as observed by the owner are enlarged lymph nodes on the outside of the body (superficial), frequently on the throat under the jaw (submandibular), on the shoulder (prescapular), in the groin (inguinal), or behind the knee (popliteal). Because lymphoma is a rapidly progressive disease, owners usually note this enlargement soon after onset, and these dogs are generally still in good body condition.

However, lymphoma can also present in a number of other ways which can be vague or even imitate other diseases. Sometimes there will be lethargy, weakness, weight loss, or digestive upset. About 10–15 percent have increased water consumption due to problems with calcium metabolism. Some will have trouble breathing, edema (fluid in the tissues), or pallor (pale gums due to anemia). Lymphoma can present in the skin and be mistaken initially for a rash. But while many other sites can be affected, enlarged lymph nodes are the most common feature.

The veterinarian’s physical examination of the dog will include checking the superficial lymph nodes, as well as internal nodes that can sometimes be felt. The abdomen will be carefully palpated for abnormalities such as enlarged liver or spleen. Most often there will be x-rays of the chest and abdomen, looking for the presence of tumor, fluid, enlarged internal nodes, or other organ involvement. Sometimes an ultrasound will offer helpful information.

A blood profile also contributes important information. Anemia is a common finding in dogs with lymphoma, and sometimes there will be atypical lymphocytes (white cells). Chemistry panels will give a good indication of the health of the liver and kidneys, a factor which may also influence decisions regarding chemotherapy. About 10–15 percent of dogs show altered calcium metabolism, which is indicative of a more aggressive form of lymphoma.

However, the diagnosis of lymphoma is not considered definitive until tissue has been obtained for microscopic examination. This can be done with a surgical biopsy, or with a needle aspirate (cytology). While the biopsy provides more complete information, cytology is usually sufficient to confirm the diagnosis. A bone marrow tap can add information which will help predict that some dogs may not do as well with chemotherapy.

When the veterinarian completes the diagnostic work-up, he/she will have categorized the stage of the lymphoma. A Stage I lymphoma is disease that appears to be confined to one site, perhaps a single lymph node. Stage I lymphomas are rare because of the rapid spread and multicentric (many



Stuart Helfand, DVM, discussed the warning signs for the onset of lymphoma.

centers) nature of the disease. Stage II lymphomas are characterized by affected nodes on only one side of the diaphragm (which separates the chest cavity from the abdomen), and also are not commonly diagnosed. The most typical presentation is of Stage III disease, in which affected nodes are found around the body. Stage IV lymphomas also show involvement of the liver and spleen; and Stage V usually indicates that the bone marrow has been infiltrated. Further, dogs are described as having “a” signs (dogs who are not acting sick) or “b” signs (dogs who are acting sick).

Staging is somewhat useful to predict which dogs may benefit most from treatment, although not enough data has been gathered on Stage I’s and II’s to be meaningful. Stage III’s and IV’s appear to do equally well, whereas Stage V’s tend to do worse. Usually dogs with “a” signs fare better than those with “b” signs.

Detailed pathology reports can also provide helpful prognostic information. While typical pathology reports only label the disease as “lymphoma,” the disease can also be differentiated into either B-cell or T-cell lymphoma. Although there are exceptions, on average, chemotherapy extends the lives of dogs with B-cell lymphomas about three times longer (>300 days) than it does for dogs with T-cell lymphomas (130–160 days). Owners faced with treatment decisions may wish to request that this typing be performed. In the future it is likely that lymphomas will be sub-typed even further, as they are in humans, where more than a dozen sub-types are known.

Unlocking the Genetic Mechanisms of Lymphoma (Modiano)

Starting with the hypothesis that mutations in tumor suppressor genes lead to a loss of function and thus contribute to the pathogenesis of lymphoma, scientists seek to answer several questions. First, they need to learn which genes are involved. Second, they will investigate whether those mutations contribute to heritable disease, to sporadic (non-heritable) disease, or, as suspected, to both.

Based upon leads suggested by the human genome project, and corroborated by information known about mouse genes, there are certain target genes which are good candidates for investigation in canines. One that was mentioned previously is the *mts-1* gene that encodes the p16 protein. p16 functions to block cells from dividing.

Another gene in which mutations can facilitate oncogenesis is *PTEN*. The PTEN protein normally plays a central role in mediating cell death at appropriate times. An example of how p16 and PTEN work together to control normal cell proliferation occurs in the vaccination cycle.

When a dog is vaccinated, lymphocytes are activated and begin dividing more rapidly. At a certain point in a normally functioning dog, a few of the activated lymphocytes will become memory cells that will respond to the actual pathogen if the dog is ever subsequently exposed. The remaining extra lymphocytes are no longer needed and will die; and the rapid division process will return to a normal baseline rate. PTEN plays a pivotal role in this cell death, while p16 inhibits the no longer necessary cell divisions. Thus, mutations that inactivate the *PTEN* gene cause excess numbers of lymphocytes to remain in circulation, while mutations that inactivate *mts-1* permit continued cell division. And the more times a cell divides, the more mutations that it accumulates, until eventually enough mutations may accumulate such that the cell becomes a cancer.

As discussed previously, pathologists are able to determine whether a lymphoma is a T-cell or a B-cell, and that contributes prognostic information based on data already available. Work already completed now also makes it possible to determine if there is a mutation of *mts-1* or *PTEN*, although it is not yet known if this information is of predictive value which might aid treatment decisions. It is hoped that data from dogs enrolled in this study will help clarify this issue.

Further, and of critical importance to breeders, will be whether these genes can help determine if there is a heritable component to lymphoma. One of the ultimate goals of this study is to determine if certain specific genetic tests can inform breeders if individual dogs are at increased risk for producing cancer-prone progeny. When and if such tests become available in the future, it is most likely that information will be expressed in terms of relative risk, rather than the absolute “clear/not clear” framework that breeders are currently more familiar with. Therefore, owners will not be “prohibited” from breeding certain dogs, while receiving a clean bill of genetic health on others. Rather, all dogs will have some degree of risk, and breeders will need to learn to make educated decisions taking into account all of the information regarding the dog’s genetic strengths and weaknesses.

Recruitment for the study began in February 2000, and includes 61 total Golden Retrievers. This number is comprised of 17 dogs with lymphoma, 29 of their unaffected relatives, and six with related diseases. Three families have more than one affected member.

In addition to the genes under investigation in the lymphoma study, several other genes are being studied in connection with other kinds of cancer. For example, it appears that the *waf-1* gene that encodes the p21 protein may be significant in melanoma and osteosarcoma. The *p53* gene has been implicated in several cancers, and may play a role in



Dr. Helfand talked with members after the seminar.

lymphoma after the initial development of the disease. That is, it is probably not important as a causative or heritable factor, but may have value in predicting the course of the disease. This may also be the case with the *RB-1* gene.

Clinical Advances in the Treatment of Lymphoma (Helfand)

As has been mentioned previously, lymphoma is a very rapidly progressive disease, and the average survival time for untreated lymphoma is only 27 days. With current chemotherapy protocols, approximately 85–90 percent of dogs will achieve a clinical remission; that is, there will be no clinical evidence of disease. Unfortunately, however, mutated cells still exist, and thus the cancer still exists. Eventually, cancer cells that are resistant to the chemotherapy drugs begin to proliferate, and the dog will relapse. Therefore, the median survival time for treated dogs is about one year (shorter for T-cell lymphomas). Although this may represent a 10 percent or more extension of the dog's life, it is not a very satisfactory outcome for a disease that strikes Golden Retrievers at a mean age of eight years old.

There are several new treatment approaches under investigation. In lymphoma, immune cells called *lymphocytes* are diseased, and one manifestation of the disease is that they are inappropriately activated. Scientists working on this disease in human medicine have discovered that these diseased lymphocytes have a protein on the surface of their cells called *the alpha subunit of an interleukin-2 receptor* (IL-2R.alpha), which is not present on normal, inactive lymphocytes. Interleukin-2 receptors (IL-2R) consist of three parts — alpha, beta and gamma subunits — but in health, only the beta and gamma subunits are expressed. The presence of the alpha subunit concurrent with the beta and gamma subunits in lymphoma is a notable abnormal feature of the disease, indicating that the lymphocytes are activated. When all three subunits are expressed, IL-2 receptors are strongly attracted to bind with one specific protein (as a key will work in a specific lock), called interleukin-2 (IL-2).

A drug called Ontak, which is composed of a poison (diphtheria toxin) attached to IL-2, has been developed for the treatment of human lymphoma. IL-2 binds to IL-2R on activated lymphocytes such as are found in lymphoma with such high affinity that Ontak behaves like a guided missile to deliver the diphtheria toxin specifically to these diseased cells. The poison is so narrowly targeted that only one molecule is necessary to kill each cancer cell. In this way, the typical systemic side effects and drug resistance problems of chemotherapy are bypassed.

There are other possible methods of lymphoma treatment that researchers have begun to examine. Because cancers are partially a result of failures of the immune system to destroy abnormal cells, one potential way to enhance or even bypass chemotherapy might be to mimic the body's natural defenses. In normal functioning, the immune system recognizes invaders such as bacteria or viruses as foreign, and produces antibodies to attack the disease-causing organism. But the immune system cannot respond to the body's own normal tissues in this way, or the result would be devastating autoimmune diseases in which immune cells destroy healthy body cells. Unfortunately, cancer cells are able to exploit this as a loophole in normal body defenses because they look too much like "self," and thereby they escape detection by the immune system.

However, it might be possible to "artificially" produce antibodies to destroy cancer cells. Using the IL-2 receptor again, and once again in the realm of human medicine, an antibody has been developed which selectively binds to IL-2R.alpha. A radioactive isotope has been attached to this antibody, so that when the antibody finds its target, radiation is delivered directly to the individual cancerous cell. There is also a commercially available antibody called rituximab that binds to mature B-cells in people. This causes the immune system to recognize these cells as foreign, and to rally natural defenses to kill them. The possibility to extend these types of treatments to dogs with lymphoma is under investigation.

Clinical Presentation and Diagnosis of Hemangiosarcoma (Helfand)

Hemangiosarcoma (HSA) is a cancer of the cells that line the interior of blood vessels, called *endothelial cells*. Typically it arises in the spleen, but is very aggressive and spreads quickly. HSA can metastasize (spread) through the blood vessel system (hematogenously), or from direct contact with loose blood ruptured into the abdominal cavity (transcoelomic implantation). In addition to metastasizing easily, HSAs characteristically are very vascular (well supplied with blood) cancers.

Most often, affected dogs are brought to their veterinarian with a history of either sudden collapse, or of cycles of weakness alternating with more normal activity. Both are a result of the internal bleeding that occurs when a tumor ruptures. Dogs who hemorrhage a large volume into their abdominal cavities and go into vascular shock are the ones who are brought in on an emergency basis. Other dogs bleed less severely, and the body is able to clot the rupture and stop the bleeding. These dogs may feel weak for a day or so, but gradually regain strength as they absorb the loose blood back into their bodies. Such episodes may repeat several times before the dog is taken to the veterinarian.

Occasionally, the veterinarian will find an enlarged abdomen on an otherwise apparently healthy dog presented for a routine examination. Or the veterinarian may be alerted to a problem by abnormalities in a blood profile. These findings may lead to further testing and diagnosis of HSA prior to a rupture.

Diagnostic tests for HSA will usually include an ultrasound of the abdomen and chest x-rays. These tests will often indicate that more than one site is involved. Unfortunately, while extensive metastases may help the owner decide not to treat, absence of obvious metastases does not offer any comfort that the cancer has not spread. Because HSA travels so easily through the body, it is generally assumed that metastasis has occurred even if not detectable. Two sites that are commonly affected are the liver and the lungs.

Blood will also be drawn for a count, biochemistry profile, and clotting profile. There is a high association between a disturbance in blood clotting mechanisms called *disseminated intravascular coagulopathy* (DIC) and HSA. This is a very serious, often fatal complication, but which can sometimes be treated successfully if detected early.

Of course, when presented with a dog in shock, the veterinarian will first attempt to stabilize the dog with blood transfusions or other medications as may be necessary. In many instances, emergency surgery to remove the spleen or other tissues involved with the tumor is necessary to control the bleeding. But whether on an emergency basis or elective, a tissue sample is necessary to definitively confirm the diagnosis.

Current treatment options for HSA are limited, and outcomes are usually unsatisfactory. Surgery to remove the tumor almost invariably leaves metastases behind. Without chemotherapy, these dogs live about three months after the surgery. Chemotherapy extends that only to about six or seven months. And often the disease is so advanced at the time of diagnosis that no treatment is possible.

Unlocking the Genetic Mechanisms of Hemangiosarcoma (Modiano)

Since current treatment protocols are so unsuccessful, scientists realize that a completely new approach is necessary. This search for alternatives, not surprisingly, begins by first trying to understand the disease on a molecular and genetic level.

During the last 10 years, there has been a great deal of research activity in the field of angiogenesis (blood vessel growth). Cancers, like all tissues, need nourishment to grow, and this nourishment is delivered by blood vessels. So in order for a tumor to grow, it needs to establish a ready blood supply for itself. It is known that cancerous tumors are able to initiate abnormal blood vessel growth for this purpose, although the exact mechanisms are not yet understood. Scientists are very hopeful that by learning more about how and why angiogenesis occurs, therapies can be developed to prevent tumors from establishing their own blood supply. Such antiangiogenesis therapies hold great promise in the treatment of many forms of cancer.

This field also ties in very nicely with HSA research. As more is discovered about normal blood vessel growth, more will also be understood about the abnormal growth that occurs in HSA. And it is likely that research into antiangio-



Members brought spirited discussion to the podium on each break, eager for more information.

genesis therapies for other cancers will have related applications in HSA.

One of the genes under investigation for its role in angiogenesis is *PTEN*. The hypothesis is that since *PTEN* is a tumor suppressor gene, loss of its function leads to unregulated growth of endothelial cells. It does this through a feedback loop using a factor called VEGF (vascular endothelial growth factor). Loss of *PTEN* function causes cells to make more VEGF, which makes them grow. Further, without the normal *PTEN* protein, the cells do not undergo normal apoptosis. Therefore, cells accumulate both by excessive proliferation and by living when they are not needed.

There are several objectives to studies currently underway involving *PTEN* in Golden Retrievers with HSA. First will be to examine whether there are, in fact, mutations in the *PTEN* gene in dogs with HSA. At this point that has proven to be the case, as *PTEN* mutations have been found in every sample examined so far. However, these mutations have been in different locations on the gene. Since the mutations are not the same, the question is raised as to whether these are occurring sporadically (non-heritable), or whether different lines may have different heritable predispositions to HSA.

Secondly, although it is now known that *PTEN* mutations exist in dogs with HSA, it must be proven that this mutation plays an important role in the disease itself. This is done by taking HSA cells that are growing out of control and actually replacing the mutated *PTEN* gene with a functional *PTEN* gene. If the mutated gene is important to the unregulated growth of the cell, then restoring its normal function should restore normal division and apoptosis to the cell.

A further objective is to determine whether detection of a mutation in *PTEN* has diagnostic or prognostic value. While most HSAs can be diagnosed with a tissue biopsy, there are some that are equivocal. In those, information about the status of the *PTEN* gene may help guide the veterinarian toward the most appropriate treatment.

Although it appears probable that *PTEN* is an important gene in HSA, it is also likely that other factors are involved

too. There are quite a number of tumor suppressor genes and oncogenes which may be investigated as the research progresses.

In addition to providing the crucial raw material for the targeted genetic research discussed above, DNA from dogs enrolled in those studies is shared with other researchers. Such collaborations make optimal use of everyone's efforts and resources. Currently, DNA is being shared with Dr. Elaine Ostrander, head of the Canine Genome Project, and Dr. Matthew Breen at the Animal Health Trust in London.

Dogs participating in all such research are identified only by code, and information regarding mutations or other genetic details about individual dogs is confidential.

Clinical Advances in the Treatment of Hemangiosarcoma (Helfand)

Tumor cells needing to establish a blood supply release substances such as VEGF, which diffuse through surrounding tissues and stimulate nearby blood vessels to grow. When the endothelial cells (these are normal cells, not HSA cells) begin to divide, they channel through the tissue in the direction of the stimulus, and hence toward the cancer cells. As the new vessels move forward, they produce a molecule on their surface which helps anchor them in the tissues in which they are growing. One of these specialized adhesion molecules is called $\alpha_v\beta_3$ (alpha.V.beta.3).

The presence of $\alpha_v\beta_3$ on endothelial cells is very significant, because it is like a red flag indicating that the cells are forming new vessels. Further, $\alpha_v\beta_3$ also plays a crucial role in preventing apoptosis; without $\alpha_v\beta_3$, the endothelial cells in the budding vessel would die. Those two characteristics of $\alpha_v\beta_3$ make it an intriguing and promising molecule to target with new anti-cancer therapies. If the functions of this molecule could be blocked, new blood vessels couldn't form to supply tumors with nutrients, and endothelial cells in budding vessels would die. Such an approach has the potential

to be important in many different types of cancer, because it does not treat the cancer per se, but rather cuts off the cancer's blood supply.

As a receptor on the surface of the cell, $\alpha_v\beta_3$ interacts with its environment by binding preferentially with certain other molecules. The substance that the $\alpha_v\beta_3$ receptor binds to has been identified as a group of amino acids called RGD (arginine, glycine, aspartic acid). RGD is found naturally in collagen. The hypothesis has been proposed that if a chemotherapy drug could be attached to RGD, RGD could be used as a vehicle to deliver the drug directly to dividing blood vessels. This offers several enormous advantages over conventional chemotherapy. By targeting the toxic drugs so specifically, body-wide side effects would be minimized. Further, it might be possible to use increased concentrations of the chemotherapy drugs, since most normal tissues would not come in contact with the toxins.

Acknowledgements

Drs. Modiano and Helfand acknowledged financial support for their research from Morris Animal Foundation, the National Institutes of Health, and the AKC Canine Health Foundation (including GRCA and numerous other breed clubs).

A special personal thank-you to Dr. Modiano and Dr. Helfand for taking time from their busy schedules to review and edit this summary (even though they did delete some of my best work, due to conflicts with material submitted for publication in scientific journals and patent applications).

Correspondence invited to: Rhonda Hovan, P.O. Box 1110, Bath, OH 44210; 330-668-0044, or fax: 330-668-0055; rhondahovan@aol.com.