Bone Marrow Transplantation: An Owner's Guide

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A Reference Guide for Bone Marrow Transplantation

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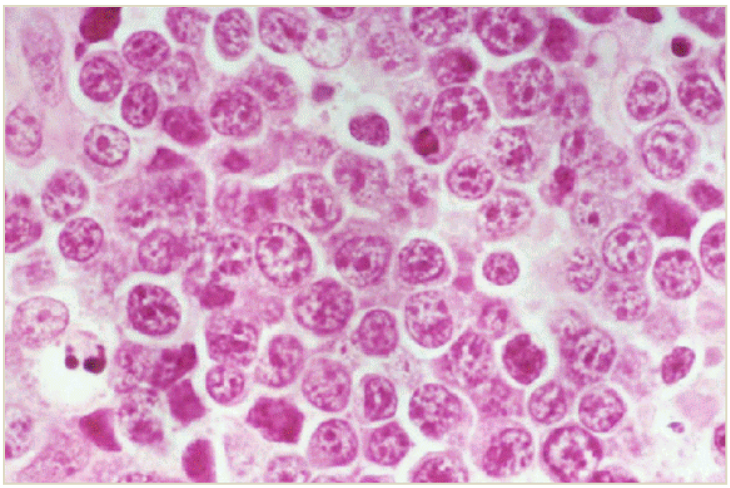
Section 1

Lymphoma

Lymphoma is a cancer that arises from a type of white blood cell called a lymphocyte. There are two main types of lymphocytes, termed T-lymphocytes and B-lymphocytes. They are essential for a healthy immune system. Their purpose is to patrol the body for harmful invaders such as bacteria or viruses. Once a threat is identified they will exponentially grow and divide, producing millions of daughter cells that will eradicate the invasion and establish a permanent defense. Because of this capacity, lymphocytes live under strict rules and constant regulation by the body to prevent uncontrolled growth. Occasionally, a mutant arises that has the ability to ignore this control. This cell and its offspring will quickly number in the billions and will have been noticed by you, in your dog, by the appearance of enlarged lymph nodes, where these cells congregate.

The mutant and its progeny have now become *lymphoma*, the most common blood cell cancer of dogs. There are two main types of canine lymphoma: B-cell lymphoma (BcL) and T-cell lymphoma (TcL). When lymphoma occurs, it will more commonly arise from B-lymphocytes; these are generally more responsive to treatment. Nonetheless, BcL in dogs is similar in disease progression and outcome to *Non-Hodgkin's Lymphoma* (that's the "bad" Hodgkin's) in people. Approximately 20% of lymphomas in dogs are of the TcL type. Due to decreased sensitivity and increased resistance to chemotherapy drugs, dogs with TcL have a much poorer prognosis than those with BcL.



TcL cells, magnification 500x (NIH)

Chemotherapy, a type of medication that is designed to kill rapidly dividing cells, is the cornerstone of the treatment of lymphoma. It is administered in the vein, (intravenous or IV) or orally (PO), depending on which drug is being given. To kill cancer cells, giving chemotherapy continuously and at very high dosages would be ideal. However, the toxicity to normal cells and hence side effects would be too severe. Therefore, breaks to allow normal cell recovery in-between each treatment

and using drug dosages based on numerous safety studies are employed. The goal of this approach is thus to improve survival but also to maintain an excellent quality of life. Unfortunately, side effects do occur. However, by allowing breaks, following strict guidelines for drug dosages, carefully evaluating each patient prior to treatment and maintaining good communication with you, these side effects, when they occur, are often minor and of a very short duration.

For lymphoma, a combination of four chemotherapy drugs, termed *CHOP-based chemotherapy*, is used. These drugs are initially given individually as intravenous and oral treatments but may later be combined. The goal of CHOP or any other multiple drug protocol is to kill as many cancer cells as quickly and safely as possible, before the cancer has a chance to develop resistance. Blocking the drug from entering the cell, quickly eliminating it before it can achieve its effect and altering the target of the drug are some of the many ways cancer cells can develop treatment resistance. Once a surviving lymphoma cell develops resistance, it will often be resistant to many chemotherapeutics; this is termed *multiple-drug resistance* (mdr). It is therefore important to use combination chemotherapy and equally important that these drugs be given on schedule and in the highest doses as safely possible. But even when these guidelines are strictly adhered to, mdr resistance, the most common cause of treatment failure in dogs, ensues.

Aside from resistance, cancer cells can overcome our best attempts to eradicate them by hiding in areas of the body that chemotherapy cannot penetrate such as the central nervous system (CNS). The ability of the body to protect the CNS is vital in the event of accidental exposure to poisons that might be encountered in every-day life. Unfortunately, however, this protective ability also creates an almost impossible obstacle when, for medical reasons, “poisons” are needed in those protected areas.

Regardless of these pitfalls, once chemotherapy has begun, approximately 80 to 85% of patients will enter an apparent disease-free state termed *clinical remission* (CR), where the lymph nodes are normal, the patient is symptom-free and no disease can be clinically detected. This state is, however, deceptive, since in nearly all cases, surviving lymphoma cells that are hidden in the body and often resistant still exist. Eventually, these cells will grow, remission will fail, and the prognosis is nearly uniformly fatal within 1-2 years.

Bone marrow transplantation, better termed *Hematopoietic Stem Cell Transplantation* (HCT), a type of treatment that has been available to human oncology for decades, has the capability to overcome these towering obstacles. It has been steadily refined over the years and offers the possibility of complete cures for lymphoma. Bringing this approach to veterinary medicine, as described below, is the hope and purpose of this protocol.

Hematopoietic Stem Cell Transplantation (HCT)

Hematopoietic Stem Cell Transplantation is designed to eliminate multi-drug resistant lymphoma cells and those in protected areas by creating a new line of attack to which they have no resistance. This takes the form of radiation therapy. Radiation therapy is the

use of high energy gamma rays to kill cancerous cells. It is similar to an X-ray with exception to the much higher energy involved, millions of electron volts (MeV). These high-energy gamma rays can be created by a *linear accelerator* (linac) or created through radioactive decay from a *Cobalt-60 therapy machine* (Co-60). To ensure that no tumor cells escape, *total body irradiation* (TBI) is performed, killing the last of the lymphoma survivors wherever they are found in the body of the patient.

The high intensity of radiation required to achieve this goal will unfortunately also eliminate normal *bone marrow hematopoietic stem cells*; these are a special group of cells that are required to produce the red cells (oxygen transport), white cells (defense) and platelets (clotting) that make up blood. Therefore, hematopoietic stem cells originating from the marrow are isolated from the patient prior to therapy, stored, and then returned to the patient after radiation is complete, where they repopulate the marrow and restore the various blood cells.

This method, consisting of the three elements of: collecting and preserving stem cells, irradiating the patient to kill tumor cells, and then replacing the healthy stored stem cells is surprisingly old in human medicine. It was first explored to preserve the lives of atom bomb victims in World War II. By 1957, the basic method described above was being evaluated for the treatment of human leukemia patients through the work of Dr. E. Donnall Thomas at Harvard, and later, at Columbia University. Throughout the 1960s and 1970s, the exact methods for radiation dosing, stem cell isolation and preservation and rules of tissue rejection were all discovered. The method of hematopoietic stem cell transplantation, then known as *bone marrow transplantation*, evolved into the standard therapy for humans with leukemia and lymphoma familiar today. In 1990, Dr. Thomas, then at the Fred Hutchinson Cancer Center, received the Nobel Prize for Medicine for his pioneering work toward the development of this therapy.

In the 1970s, it was also recognized that malignant lymphoma in dogs was a close analogue to the human form of the disease, and the chemotherapy regimens used in people began to be applied to canine medicine as well. Ironically, even though canine models had been used to establish the HCT system in people, the therapy itself has not been previously available in veterinary medicine. The primary reason has been cost: for example, the gene marker and DNA tests used in humans to detect tumor cells would originally easily have cost $1000 *per test*. However, in the last ten years, this type of testing has become common in both human and veterinary medicine, and costs have dropped greatly, mostly due to advances in DNA technology.

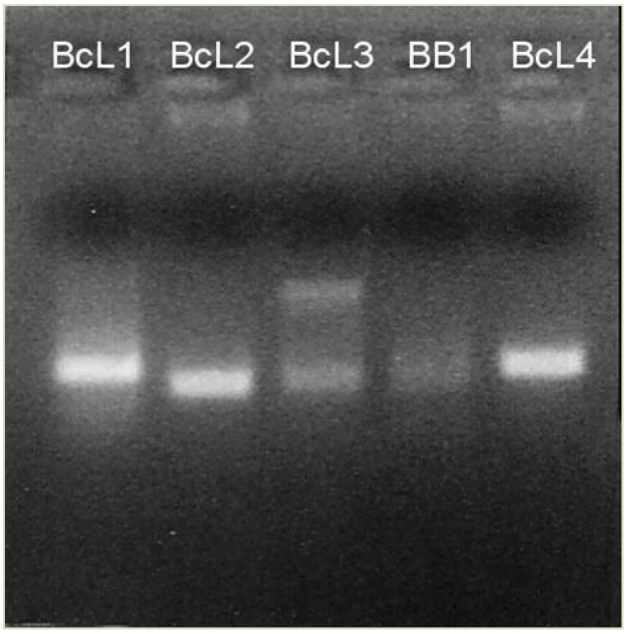
The machines used for isolating stem cells from the patient, termed *apheresis* machines, are also becoming more common in a veterinary practice, having various other therapeutic uses. Finally, radiation oncology in veterinary medicine, with its associated linear accelerator machine or Co-60 machine, can also now be found in a few select practices across the country. This convergence makes it possible to offer HCT as a veterinary procedure for the first time.

Section 2

The First Two Steps: Staging and Consolidation Therapy

The HCT procedure has 5 major clinical phases or steps. The first two steps (**staging** and **consolidation**) are *check-points* through which a dog must successfully pass before proceeding to **apheresis**, **radiation therapy,** and finally, the **recovery** phases of the protocol.

Staging, prior to beginning CHOP, diagnostic testing will be performed, including chest X-rays, abdominal ultrasound, and a DNA test called PCR (polymerase chain reaction). The goal of this series of tests is to establish a baseline on the extent and location of the cancer so that it can be followed throughout subsequent treatment. If, with follow-up examinations and repeat testing, no cancer is detected, a *clinical remission* (CR) has been achieved. After CR has been achieved, and the DNA-based tests are also negative for cancer cells, the patient is termed to also be in *molecular remission* (MR) and can then proceed to consolidation.



PCR detection of B-cell Lymphoma DNA in the blood of five patients: BcL3 is a CR but not MR, BB1 is CR and MR, the rest are not in remission. (Engene Corp)

Consolidation, step two, is the same method employed in human medicine; the purpose of consolidation is to further reduce any hidden cancer cells in the body and bone marrow, and to ensure that none will be present in the stem cell preparation on apheresis day. High-dose chemotherapy using cyclophosphamide (cytoxan) is used in this phase. Because cytoxan can irritate the urinary bladder, your dog will stay overnight for monitoring and for the administration of intravenous medications that will protect their bladder from damage. Over the next two weeks, blood samples will be evaluated to insure the patient's white blood cell and platelet levels return to normal. Once the dog's blood cell counts are normal or approaching normal, the PCR blood test will be repeated. If no cancerous cells are found, the patient is confirmed to be in molecular remission (MR) and can then proceed to step three, **apheresis**. A molecular remission is considered to be the best-case scenario under which to proceed to perform stem cell therapy.

At this time, oral antibiotics are initiated to lower your dog's gastrointestinal bacterial numbers (termed "sterilizing the gut"). This will minimize the chance that bacteria from the intestinal tract will gain entrance to the patient's bloodstream, causing a systemic infection called sepsis. The antibiotics are maintained from this time until just prior to release from the hospital. If the dog has evidence of infection anywhere in their body, such as the urinary tract, the oral cavity, or skin surface, these infections must also be detected and treated prior to proceeding to the **apheresis** step of the protocol.

Step Three: Apheresis

Your dog is now ready to undergo harvest of their hematopoietic stem cells using an apheresis machine. This machine is a special centrifuge that separates cells based on size (stem cells are much bigger than red cells). The patient's blood is passed through the centrifuge where the larger stem cells are collected and saved. The rest of the blood and its components are returned to the dog in a cyclic process. In order to enrich their blood with abundant stem cells to be collected, your dog will be admitted to the hospital the evening before the procedure. In the very early morning, on the day of apheresis, the dog will be given an injectible medication subcutaneously (under the skin) to stimulate the release of the stem cells from bone marrow into their bloodstream.

Approximately 6 hours later, the stem cells are ready for harvest. Apheresis is not a painful procedure; however, it is imperative that movement be minimized. Therefore, the patient is anesthetized for the 3 to 4 hour procedure.

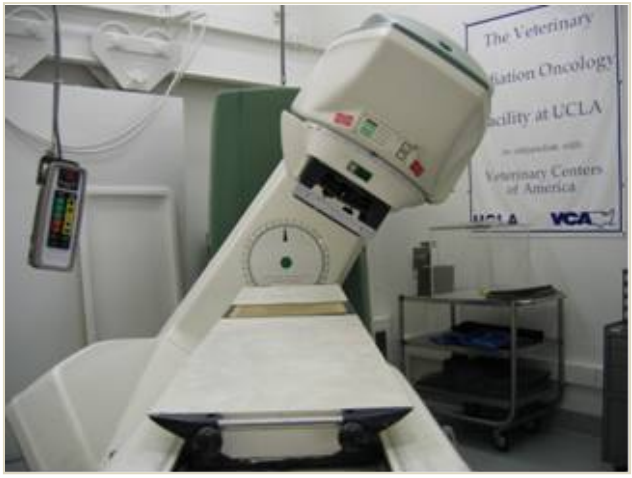
The apheresis process itself consists of placing the patient on a comfortable bed, attaching the apheresis machine to a specialized catheter that has been placed earlier that day, and then collecting the stem cells from the patient.

Cobe Spectra Apheresis Machine (Caridian Corp.)

Samples of the harvested hematopoietic stem cells will be sent out overnight for analysis to two separate laboratories. These tests will determine whether adequate stem cells have been collected by the apheresis procedure and that no contaminating tumor cells are present in the harvest. The patient will remain in the hospital overnight, in preparation for either a second round of apheresis (though this should not be necessary in most cases), or

radiation therapy the following day. If adequate, tumor-free stem cells have been collected, the patient may now proceed to step four, **radiation therapy**.

Step Four: Radiation Therapy



Cobalt-60 veterinary radiation machine at UCLA

On this day, the day after apheresis, your dog will receive two 70-minute treatments (these are termed *fractions*) of total body radiation with a three-hour rest period in between. The radiation therapy is performed off site on the UCLA campus in a dedicated VCA West Los Angeles Animal Hospital radiation oncology facility. Radiation patients are transported by our staff to the site located 1.25 miles north of our main hospital. This off-site center is fully equipped and at minimum is staffed by a veterinary radiation oncologist, a registered veterinary technician, an experienced animal handler, and a human-registered radiation therapist. This staff will ensure your dog is properly cared for during treatment.

Radiation therapy is not painful. However, for proper patient positioning, the dog must be placed under anesthesia. Once the two fractions have been given, the previously collected, (non-irradiated) hematopoietic stem cells are returned to the dog intravenously. Once in the blood stream, these stem cells will migrate into the bone marrow, settle in, and begin to grow. Your pet will now proceed to the final step, **recovery**.

Step Five: Recovery

This last phase is a critical step that will last approximately 2 weeks. During this time, blood cells, including any remaining cancer cells, will be dying as a result of the radiation treatment. At the same time, the non-irradiated, harvested stem cells that were returned to your dog will begin to grow. The old white cells, and platelets, will die at a slightly faster rate than the new stem cells can replace them. This creates a *nadir,* or lowest level in the blood, approximately 7 to 10 days after radiation therapy. During this time, the patient's immune system is therefore weak and they are also at risk of bleeding from low platelet numbers. Recovery will quickly follow as the new hematopoietic stem cells will shortly thereafter produce enough white cells and platelets to restore your dog's blood cell counts back to normal.

Beginning the day of radiation, food and water will be withheld for 2 to 3 days to lessen the risk of intestinal irritation. During this time, they will receive intravenous or subcutaneous fluids as needed to maintain health. Beginning 24 hours after radiation therapy, the patient will be placed into a special isolation housing area. This is a carefully monitored, comfortable hospital ward equipped with dimming lights, a flat screen TV, and a stereo sound system. For sterility and for your dog's protection, this suite is separate from all other hospital patient areas, and especially from contact with other dogs.

Your dog will be monitored 24 hours a day while in isolation to ensure that they remain comfortable during their stay. Daily blood testing and physical examination will be performed to monitor organ function and to detect their *nadirs*.

The expected duration of time that the patient will be in isolation is 10 days (or until the patient's white blood cell count reaches a safe level). During that time, daily visitations by family will be allowed, as long as strict sterile technique (operating room standard) is maintained. During the period of isolation, twenty-four hour veterinarian care is maintained, and the patient will be attended to by the most experienced veterinary nurses, using the most advanced sterile techniques to maximize the patient's comfort and safety.
If needed, aggressive antibiotic and anti-fungal therapy to treat infection, blood and/or platelet transfusions to treat bleeding or anemia, and aggressive anti-nausea medications to treat gastrointestinal upset will be given.

Once your dog's white blood cell count returns to a safe level ($\geq$1000 neutrophils/µl) they will be allowed out of isolation. However, because platelet counts take slightly longer to recover, they will remain hospitalized with us for a few more days to ensure that they are kept calm and unexcited. This will help to conserve any remaining platelets and to prevent injury. During these few remaining isolation days, they will continue to be carefully monitored to ensure they remain safe and comfortable. As soon as their platelets numbers return to a safe level, they will be discharged for further home care.

Post Hematopoietic Stem Cell Transplant: Follow-Up

Your dog's platelets may take an additional 7 to 14 days to return to normal. No medication will be necessary at this time. However, their activity will need to continue to be restricted and they will be required to return to us or you local veterinarian for periodic blood cell counts until all parameters have normalized.

Congratulations, the protocol is complete! If the treatment is successful, no further chemotherapy, or any other tumor treatments, should ever be required. To ensure a long, healthy and cancer-free survival' we will continue to monitor your pet. We recommend they return once monthly for the first six months, and then every two months until they reach a full year post-treatment. After one year, rechecks will be scheduled on a quarterly basis. In addition to the thorough physical examinations performed at each visit, chest X-rays, abdominal ultrasound and PCR testing will be performed 2 to 4 times yearly.

Summer 2006

VETERINARY executive report

Dog stem cell transplant puts WSU on world stage

CNN: procedure video among its most requested

The image of a sleeping beagle captured the attention of the nation, as Bailey rested quietly after undergoing a rigorous stem cell transplant procedure. The six year old Beagle was brought to WSU's College of Veterinary Medicine for full body radiation, vital in helping treat her lymphoma.

"It's exciting, but somewhat nerve wracking," said **Dr. Pat Gavin '71**, a veterinary radiation oncologist with WSU. "You take those extra steps to ensure this first case goes smoothly, but it's exciting in that this really is groundbreaking in the treatment of cancer in pets."

Bailey was brought here by **Dr. Ed Sullivan**, a veterinarian from Bellingham who completed the very first stem cell procedure worldwide for a client animal. "This was no easy task," said Dr. Sullivan, adding, "the real challenge came in finding one of Bailey's siblings who was a perfect match for a stem cell treatment."

Much of the work in using radiation treatment for animals was pioneered at WSU by Dr. Gavin. For him, it's just the latest chapter in advancing care. "With lymphoma right now there is no cure, in both animals and humans, unless you get pretty aggressive, so for animals this is a whole new area with plenty of promise."

Bailey rests comfortably after procedure

At last check, Bailey's recovery continues. The story was picked up by television stations in Seattle, and ultimately CNN. By mid-afternoon CNN's Web site showed the story to be among its more requested videos in the country.

First Report of Stem Cell Transplant (University of Washington Newsletter, 2006)

Section 3

Lymphoma and Stem Cell References

General reference on canine lymphoma:
www.wikipedia.org search phrase: "lymphoma in animals"

General reference on bone marrow transplantation:
www.wikipedia.org search phrase: "hematopoietic stem cell transplantation"

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Hospital Department of Oncology

Our stem cell transplant team includes veterinarians and registered veterinary technicians who are specialists trained in the field of apheresis, medical oncology, and radiation oncology. We are supported by a large staff of professionals in one of the largest referral hospitals in Southern California.

Medical Oncology
- Johnny D. Chretin, DVM, DACVIM (O)
- Trina Hazzah, DVM

Radiation Oncology
- Travis Tuchak, DVM
- Jesus Jocobo, RT(T)

Oncology Staff
- Stephanie Cantrell, RVT, Senior Technician
- Spike Vainshtein, RVT, Medical Technician
- Regina Estrada, RVT, Medical Technician
- Marwin Gillett, Oncology Liaison

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Frequently Asked Questions: (FAQ)

What is the chance that my dog will achieve a cure following total body irradiation and stem cell transplantation?

In human medicine, approximately 40 to 60% of patients with lymphoma or leukemia can expect to achieve a cure. At this time, 23 canine patients have been treated with this protocol at 2 separate veterinary centers. The results obtained are sufficient to determine that the procedure can be performed safely, and the current cure rate, while it is still too early to know definitively, is matching or exceeding human rates.

If my dog achieves a clinical remission (CR) but not a molecular remission (MR), will he or she still be eligible for stem cell transplantation?

In human medicine, the procedure goes forward even if the stem cell harvest is found to have a few contaminating tumor cells. But as one might expect, the deeper the remission, and the fewer contaminating tumor cells present, the better the outcome. Therefore, a patient who achieves a CR but not an MR might still be eligible to proceed with the stem cell transplant protocol, but it is possible that such patients will have less of a chance to achieve a cure.

Are there any factors that could exclude treatment of my dog with this protocol?

Patients that are less than 5 kg (these are hard to apherese) or have significant organ dysfunction (liver, kidneys, or heart) are not eligible. Additionally, patients who are diagnosed with uncontrolled infections or who are at high risk of developing infections secondary to other disease (e.g. patients with diabetes mellitus or Cushing's disease) may also not be eligible.

Further, failure at any of the checkpoints (which are: achieving complete remission on the initial CHOP chemotherapy, achieving molecular remission at any point, and achieving a cancer-free stem cell harvest) may also preclude going forward with the protocol.

What is the expected mortality rate of the procedure itself?

To date, there has been only one clinical veterinary patient (out of 23 treated cases) who has died immediately following the HCT procedure, due to infection. This patient's status had been considered to be very high risk before entering into the HCT program. In dogs, as in humans, great strides have been made to reduce complications of this type, mainly due to 24 hour monitoring of the patient, and prompt response with powerful antibiotics.

What are the possible complications associated with the HCT protocol?

Common complications include diarrhea and nausea (treated supportively via use of fluid support, anti-diarrheal and anti-nausea medications) in the days immediately post-treatment.

Less common side effects include anemia secondary to bleeding (treated with transfusions), bacterial infections (treated with aggressive antibiotics), and prolonged nausea (treated with anti-nausea medication).

Rare side effects include severe bladder irritation (cystitis) secondary to treatment with high dose cyclophosphamide (treatment is supportive for discomfort, usually self-limiting within days to weeks, rarely persists for months), formation of blood clots, called emboli, in areas of the body such as lungs or brain (treated with heparin), and unusual infections from fungal or parasitic organisms (treated with organism-specific medications).

There is the possibility of failure of the stem cells to grow in the patient's bone marrow after they are returned. This complication is more common when stem cells from one dog are being used to treat a different dog such as a littermate (*allogeneic* transplant). We are not performing allogeneic transplantation in this study. Rather, we are obtaining the hematopoietic stem cells from the patient and then returning them to the same patient (termed an *autologous* transplant). As a precaution to this unlikely event, we prophylactically save a portion of the original stem cell harvest. Should the growth of stem cells (*engraftment*) take longer than expected or not occur we would administer the second treatment.