HEMATOPOIETIC CELL TRANSPLANTATION

An Owner's Reference Guide for Bone Marrow Transplantation

Bone Marrow Transplant- An Owners' Guide
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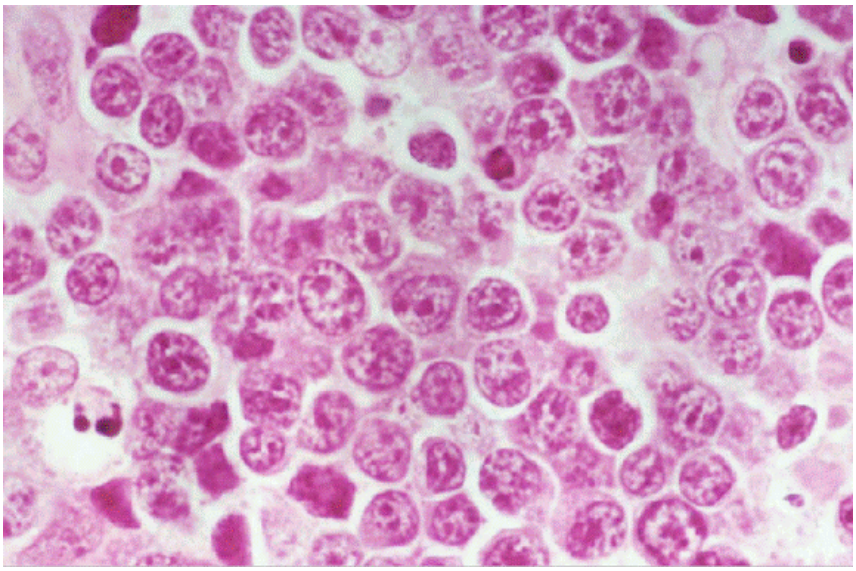
Section I.

Lymphoma

Lymphoma is a cancer that originates from one type of the white cells of the blood, termed *lymphocytes*, which normally patrol for invaders and destroy them. When a normal lymphocyte encounters its target, it divides and grows, producing millions of daughter cells to help fight the infection. Because they have this capacity, lymphocytes live under strict rules and constant regulation by the body to prevent uncontrolled growth. Occasionally, a mutant arises with the capacity to ignore this control, and continues to divide. This cell and its offspring 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 have congregated. They may also accumulate within the gastrointestinal tract.

This mutant and its progeny have now become a *Lymphoma*. Although the majority of canine lymphomas arise from B-lymphocytes (these make antibody), this cancer can also arise from a T-lymphocyte (these fight viruses and cancer) and are abbreviated BcL and TcL respectively. The BcL condition in canines is equivalent to *Non-Hodgkin's Lymphoma* (the "bad" Hodgkin's) in people, with similar disease progression and outcomes.



TcL Cells, magnification 500x (NIH)

Standard therapy in dogs for lymphoma employs a combination of chemotherapy drugs, usually termed *CHOP*, where each letter stands for one of the drugs employed. By attacking the lymphoma with a series of different drugs, this plan is designed to prevent the lymphoma from developing resistance. Unfortunately, in most cases, lymphoma can thwart this approach by developing *multi-drug resistance* (mdr) after which the surviving

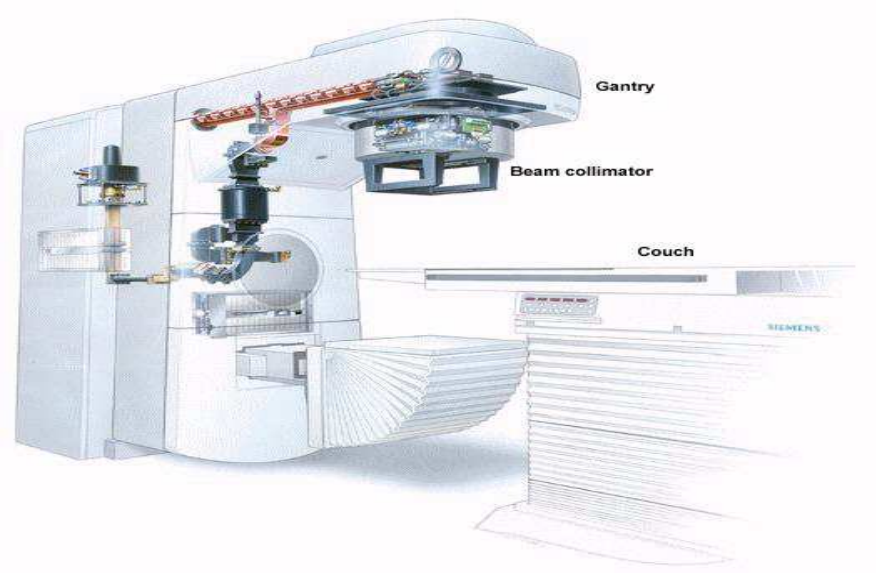
cells learn to block the drugs from entering their cells, or can destroy them soon afterwards. Another approach used by resistant cancer cells involves "hiding" in sites in the body where chemotherapy cannot penetrate; as a former B or T cell, the lymphoma has privileged access everywhere in the body that another cell type would not have.

Soon after chemotherapy has begun, the patient will enter an apparent disease-free state termed *clinical remission (CR)*, where the lymph nodes are normal or the "mass" goes away, and no disease can be clinically detected. This is deceptive, since, in nearly all cases, surviving mdr lymphoma cells still exist, hidden in the bone marrow or blood; eventually, these will grow, remission will fail, and the prognosis is nearly uniformly fatal within 6 months-2 years. A similar progression exists for people, with a slightly longer time-line of 3-5 years.

However, in human medicine, a form of therapy termed *Bone Marrow Transplant* (BMT) has been steadily refined, 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.

Bone Marrow Transplant (BMT)

Bone Marrow Transplant is designed to circumvent multi-drug resistance in lymphoma cells by creating a new line of attack to which they have no resistance. This takes the form of radiation therapy, which kills the last of the lymphoma survivors; wherever they may be found in the body, with high-intensity gamma rays, delivered by a device termed a *linear accelerator* (linac). Since the radiation beam encompasses the entire body to ensure that no tumor cells escape, this therapy is called *Total Body Irradiation* (TBI).



Linear Accelerator Machine (Siemens Corp)

However, the intensity of radiation required to kill all the cancer will also eliminate *bone marrow 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, to preserve them, stem cells originating from the marrow are isolated from the patient prior to radiation 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: preserving stem cells, irradiating the patient to kill tumor, and then replacing the stored stem cells, is surprisingly old in human medicine. It was first explored to preserve the lives of A-bomb victims, and by 1957, the basic method described above was pioneered for human leukemia patients by 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 for tissue rejection were all developed, and the method evolved into the standard human therapy for leukemia and lymphoma used today. In 1990, Dr. Thomas received the Nobel Prize for Medicine for his contribution to the discovery of this method.

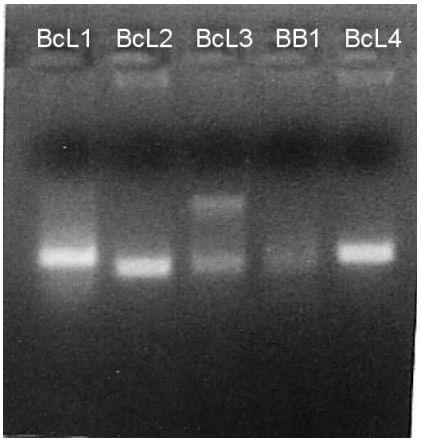
In the 1970s, it was also recognized that malignant lymphoma in dogs was a close analogue to the human form of the disease, and the chemotherapy regimens used in people began to be applied to canine medicine as well. Ironically, even though canine models had been used to establish the BMT system in people, the therapy itself has not been previously available in veterinary medicine. The primary reason has been the availability of centers of excellence like the Veterinary Specialty Center of the Hudson Valley to perform the procedure (www.vschv.com) and advances in DNA technology.

Section II.

The BMT procedure has 5 major clinical phases, each of which will be discussed below. The first two stages are *check-points*, through which the dog must successfully pass before proceeding to radiation therapy and recovery.

Staging and Consolidation Therapy

The term “staging” refers to the examination for the degree of cancer in your dog’s body or any other disease, and to the establishment of DNA-based tracking tests to monitor the remission. Pre-BMT chemotherapy can be done here at VSCHV or through your primary care veterinarian or another oncologist or internal medicine specialist. There must be advanced communication between our BMT team and your referring doctor for this to be effective and successful. After chemotherapy (CHOP) is complete, and prior to progressing to consolidation therapy, your dog will undergo various clinical tests here at VSCHV, to detect any residual cancer cells. If none are detected, a *clinical remission* (CR) has been achieved. If the DNA-based tests are also negative for cancer cells, the patient is termed to be in *molecular remission* (MR), and the patient can then proceed to consolidation therapy.



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, and the rest are not in either form of remission. (Engene Corp)

Consolidation therapy uses the same method employed in human medicine; the purpose of consolidation is to reduce further any hidden cancer cells in the body, or marrow, and to ensure that no cancer cells are present to contaminate the stem cell preparation on apheresis day. High-dose chemotherapy using cyclophosphamide (cytox) is used in this phase; the patient will stay overnight for monitoring and be released in the morning. Over the next week, blood samples will be sent to a specialized lab that performs PCR, a DNA test designed to be so sensitive as to be able to detect even a single remaining cancer cell in the blood. If none are found, the patient is now in molecular remission (MR) and can proceed towards the apheresis step. A molecular remission is considered to be the best case scenario under which to proceed to Bone Marrow Transplant.

Also, in preparation for the radiation therapy to come, oral antibiotics are initiated at this time to lower the bacterial numbers in the gastrointestinal tract. This will minimize the chance that bacteria from the intestines will gain entrance to the bloodstream, after the radiation therapy, causing a systemic infection called sepsis. These antibiotics are maintained from this time until just prior to release from the hospital. If the patient has evidence of infection anywhere in the body such as the urinary tract, the oral cavity, or the skin surface, these infections must be detected and treated prior to proceeding to the next phase of the protocol.

Apheresis Day

The dog is now ready to undergo harvest of stem cells using an apheresis machine under seadation/anesthesia. This machine is basically a special centrifuge that separates cells on the basis of size (stem cells are much bigger than red cells). These are purified and the blood returned to the patient in a cyclic process. In order to enrich the blood with abundant stem cells to collect, the patient will be admitted to the hospital the evening

before apheresis, and, in the very early morning, will be given a medication to stimulate the release of the stem cells from the bone marrow into the blood.

Approximately 6 hours later, the blood is ready for harvest and the dog will be transported to the appropriate section of the hospital to initiate apheresis. The patient is sedated/anesthetized for the process as a precaution to keep the dog still, but the procedure itself is painless.

The apheresis process itself consists of placing the patient in 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. This procedure takes 3 to 4 hours in total. A portion of the collected sample will be sent by FedEx to a facility for analysis. This analysis will determine whether adequate stem cells have been collected by the apheresis procedure. Another sample will be sent to the DNA analysis laboratory to make sure the stem cells are not contaminated with tumor cells. The patient will remain in the hospital overnight, in order to be ready to proceed with either a second round of apheresis (though this should not be necessary in most cases) or radiation therapy. If adequate, tumor-free, stem cells have been collected, the patient may proceed to radiation therapy.

Cobe Spectra Apheresis Machine (Caridian Corp)

Radiation Therapy Day

On this day, 1-4 days after Apheresis Day, the patient will receive two 70-minute courses of x-ray radiation therapy, with a 3-hour rest period in between. During the periods of irradiation, the patient will be sedated/anesthetized to ensure proper positioning and maintain complete stillness, but no discomfort will occur. Immediately after the final radiation dose, the previously collected stem cells are returned to the patient i.v., where they then immediately migrate to the marrow, settle in, and begin to grow.

Post-Treatment Recovery

This phase is critical-lasting approximately 2 weeks. During this time, the stem cells returned to the body will divide and replace blood cells; also during this time, old blood cells and tumor cells will be dying out. The old white cells will die out just a bit quicker than the stem cells can get established and make more; this creates a white cell *nadir* or lowest level in the blood. During this time, the patient's immune system is weak and cannot fight infection, and occurs from 7-10 days after radiation therapy. After that, the new stem cells have produced enough white cells for a quick recovery in numbers, and the immune system is restored.

Starting on the night of radiation therapy, therefore, the patient will be placed into the ICU. Patients will receive fluids by intravenous administration, but will not be allowed to take in oral food or water for several days. It is unlikely that the patient will desire food during this time, in any case, as the GI tract experiences some irritation during the first few days following radiation therapy.

The patient will be monitored several times daily via physical examination and observation of vital signs. Daily blood testing will be performed to determine that normal organ function is maintained, and to detect the white cell *nadir*.

The patient will be expected to remain in hospital for 15-20 days (or until the patient's white blood cell count reaches a safe level). As the white blood cells begin to drop, the patient is moved to a carefully monitored, comfortable hospital ward, separate from all other hospital patient areas, and especially from other dogs. During that time, daily visitations by the patient's family will not be allowed---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 the patient develops a fever or other signs of infection during hospitalization, aggressive antibiotic therapy will be initiated to counter this infection. If bleeding, secondary to a low platelet count, occurs one or more blood transfusions may be required to prevent anemia. Any gastrointestinal upset will be managed with appropriate medications, hydration, and nursing care.

Once your dog's white blood cell count is determined to have returned to a protective level, it will take a few days for platelet counts to return to normal: these are the cells that control clotting, so the patient must be kept quiet and unexcited during this phase. To help the dog through this phase, a platelet transfusion may be administered. Soon the patient can leave the isolation area and be transferred back to the ICU. Several times a day, physical examinations and vital monitoring will continue, and the patient can leave the hospital after all blood work is normal, usually 14 days after the radiation treatment day.

Out-Patient Follow-up:

After the patient is able to leave the hospital, periodic blood cell counts will be monitored back at VSCHV in the weeks to follow. Clinical remission will be monitored once monthly via physical examination and PCR (MR) and once every 3 to 4 months via chest X-rays and abdominal ultrasound. No further maintenance chemotherapy, or other conventional tumor treatments will be required if the treatment is successful.

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 Bone Marrow Transplant (University of Washington Newsletter, 2006)

Section III.

Frequently Asked Questions: (FAQ)

>*What is the chance that my dog will achieve a cure following total body irradiation and Bone Marrow Transplant?*

In human medicine, approximately 40 to 60% of patients with lymphoma or leukemia can expect to achieve a cure following total body irradiation and Bone Marrow Transplant. 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, predicted human rates.

>*If my dog achieves a clinical remission (CR) but not a molecular remission (MR), will he or she still be eligible for Bone Marrow Transplant?*

In human medicine, the procedure goes forward even if blood or marrow samples are found to have a few contaminating tumor cells. But as one might expect, the deeper the remission, and the fewer the tumor cells in the blood, the better the outcome. Therefore, a patient who achieves a CR but not an MR might still be eligible to proceed with the Bone Marrow 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 with a body size of less than 15 kg (these are difficult to apherese), have organ dysfunction (heart, kidney, liver), who are diagnosed with uncontrolled infections, or who are high risk to develop infections secondary to other disease, (for example, diabetes mellitus, or adrenal disease such as Cushing's disease), will not be eligible for Bone Marrow Transplant. Also, failure at any of the checkpoints, which are: achieving CR (CHOP), MR (consolidation) or the isolation of a useful, tumor-free, stem cell harvest, will 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-1/1/10) who has died immediately following the BMT procedure, due to infection. This patient's status had been considered to be very high risk before entering into the BMT 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 BMT protocol?*

Common complications include diarrhea and nausea (treated supportively via use of fluid support and Imodium) 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), formation of blood clots, called emboli, in areas of the body such as the lungs or brain (treated with heparin),

and unusual infections from fungal or parasitic organisms (treated with organism-specific medications).

Finally, there is the possibility of failure of the stem cells to grow in the patient's bone marrow after they are returned post-TBI. This is a complication seen more with transplants in human medicine using donor stem cells from another person (termed *allogeneic* transplants), and is not the method used in this procedure (known technically as an *autologous* transplant). For our patients, a rescue plan in which extra stem cells are frozen in storage will be used, as the apheresis procedure typically yields more cells than are necessary for the Bone Marrow Transplant. These extra cells will be thawed and administered to the patient in the unlikely event that the initial engraftment is slow, or does not take place.

References

Advanced References

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