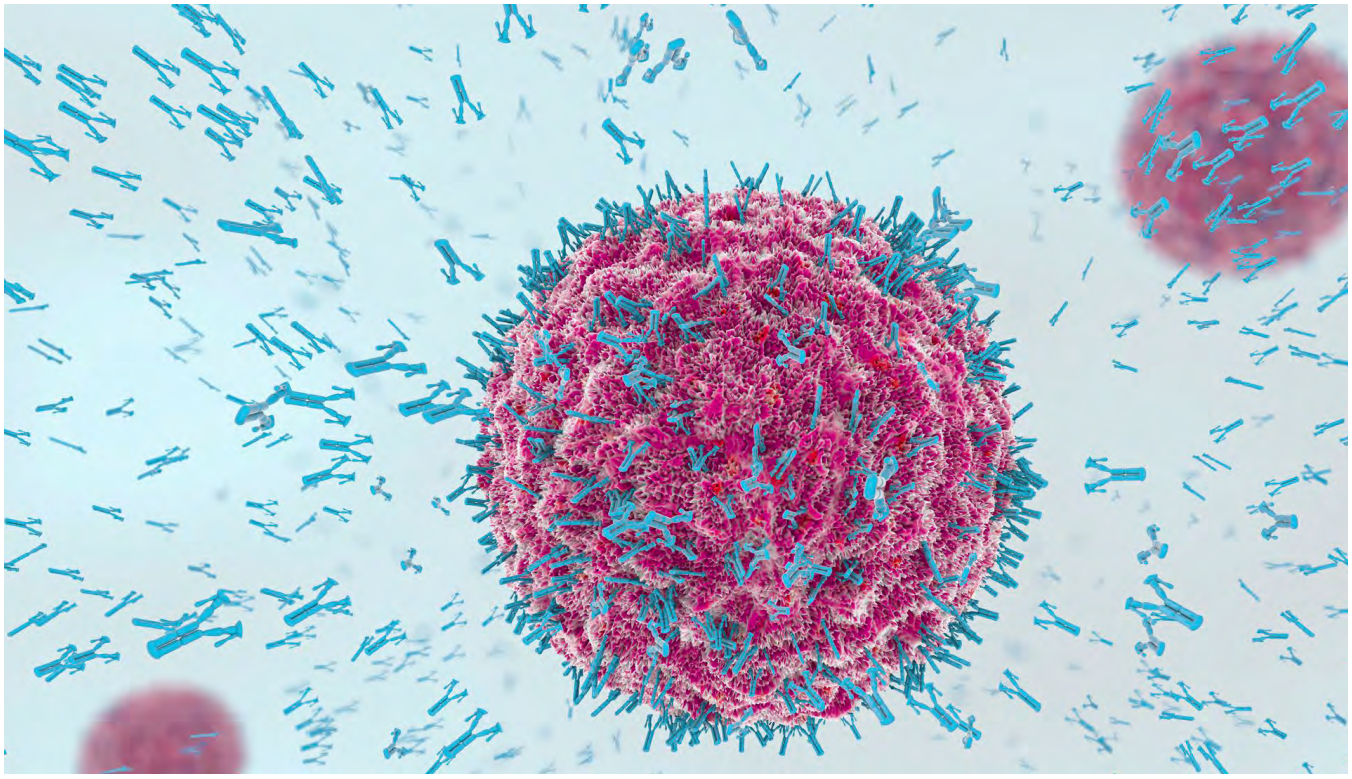
STEM CELL THERAPY:

AN OWNERS' GUIDE

A REFERENCE GUIDE

FOR BONE MARROW TRANSPLANTATION



A Reference Guide for Bone Marrow Transplantation

Stem Cell Therapy: An Owners' Guide

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TOC

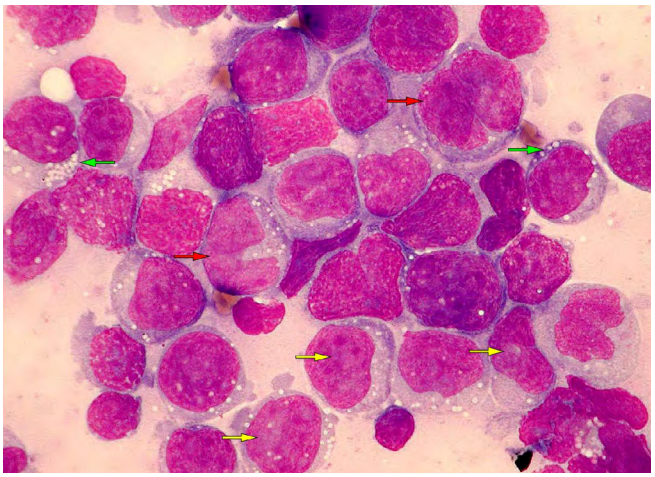
01
LYMPHOMA

LYMPHOMA: CAUSES AND CURRENT THERAPIES

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.

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.

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



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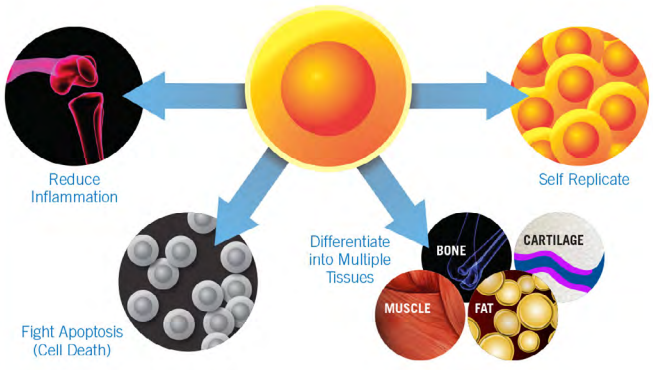
with a series of different drugs, this plan is designed to prevent the lymphoma from developing resistance. Unfortunately, in most cases, lymphoma can thwart this approach by developing multi-drug resistance (mdr) after which the surviving cells learn to block the drugs from entering their cells, or 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, 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 1-2 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 Stem Cell Therapy (SCT) 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.



STEM CELL TRANSPLANTS: THEORY, HISTORY AND PRACTICE

Stem Cell Therapy 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).

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 SCT 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, can also now be found in a few select practices across the country. This convergence makes it possible to offer SCT as a veterinary procedure for the first time.

02

STEM CELL TRANSPLANT PROCEDURE

The SCT 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

The term 'staging' refers to the examination for the degree of cancer in your dog's body, and to the establishment of DNA-based tracking tests to monitor the tumor. After CHOP is complete, and prior to progressing to consolidation therapy, your dog will undergo various clinical tests, including chest X-rays and abdominal ultrasound, 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.

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 stem cell therapy.

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. 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 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.

COBE SPECTRA APHERESIS MACHINE (CARIDIAN CORP)

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 the next morning. If adequate, tumor-free, stem cells have been collected, the patient may proceed to radiation therapy.

RADIATION THERAPY DAY

On this day, the day after Apheresis Day, the patient will receive two 70-minute courses of gamma-radiation therapy, with a 3-hour rest period in between. During the periods of irradiation, the patient will be sedated 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 a critical phase 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 special isolation housing ward. This is a carefully monitored, comfortable hospital ward, separate from all other hospital patient areas, and especially from other dogs. 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 isolation for 10 days (or until the patient's white blood cell count reaches a safe level). During that time, daily visitations by the patient's family will likely be allowed, as long as strict sterile technique (operating room standard) is maintained. During the period of isolation, twenty-four-hour veterinary 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 occurs secondary to a low platelet count, one or more blood transfusions may be required to prevent anemia. Any gastrointestinal upset will be managed with appropriate medications, hydration, and nursing care.

The isolation ward is also designed to provide your dog with the most comfort possible and with varied diversions (TV, radio, and frequent play visits) during his or her stay.

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 to a regular patient care ward. 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

Out-Patient Follow-up: After the patient is able to leave the hospital, periodic blood cell counts will be monitored in the weeks to follow. Clinical remission will be monitored once monthly via physical examination and once every 3 to 4 months via chest X-rays and abdominal ultrasound. Molecular remission will be monitored once every 3 to 4 months via blood sampling. No further maintenance chemotherapy, or other conventional tumor treatments will be required if the treatment is successful.

03

APPENDICES & REFERENCES

FREQUENTLY ASKED QUESTIONS: (FAQS)

What is the chance that my dog will achieve a cure following total body irradiation and stem cell therapy?
In human medicine, approximately 40 to 60% of patients with lymphoma or leukemia can expect to achieve a cure following total body irradiation and stem cell therapy. 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 stem cell therapy?
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 stem cell therapy 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 stem cell therapy. 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) who has died immediately following the SCT procedure, due to infection. This patient's status had been considered to be very high risk before entering into the SCT 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 SCT 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 stem cell therapy. 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.

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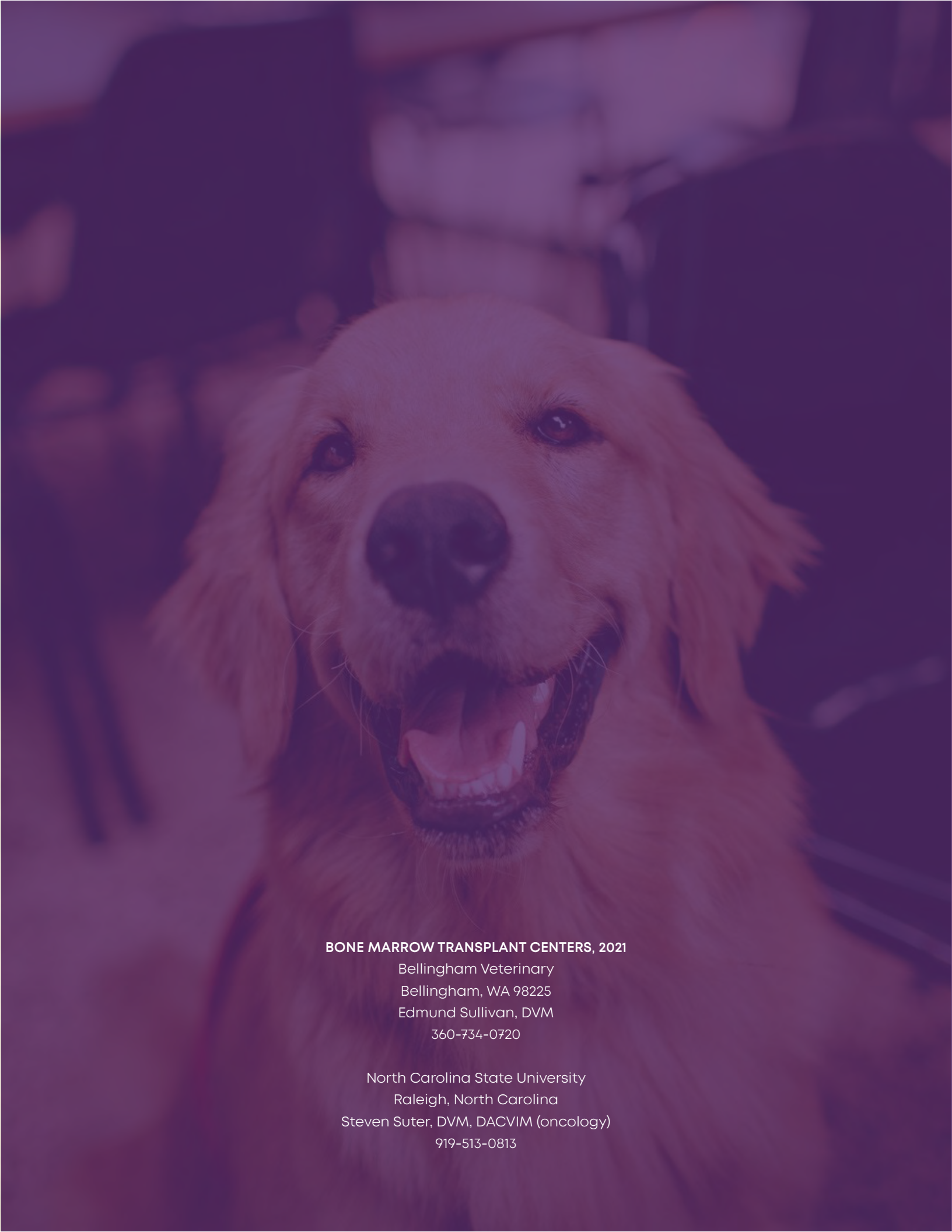

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